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INTRODUCTION.

The deterioration of food products has assumed an added significance during the war which it seems likely to maintain. This applies to sugar for the familiar reasons as well as the complicating problems of equalization, distribution and reserve. The problem of sugar deterioration has long been the subject of investigation and since the original observations of Payen[33] and Dubrunfaut[11], it has been considered to be of a microbiological nature. The contributions in this field have been comprehensively reviewed by Owen[29 30 31 32] and others, so that it is unnecessary at this point to do more than note that the emphasis in such research has been, to a great degree, on the bacterial flora involved, as evidenced by the valuable contributions of Lewton-Brain and Deerr[5], Deer and Norris[9], Dodson[10], Greig-Smith[19], Fermi and Montesano[16] and Greig-Smith and Steel[21]. More recently, however, there has been an increasing tendency to regard the fungi (or molds, as they are more commonly termed) as playing an important rôle in the inversion of sucrose which occurs in manufactured cane sugar.

Since comparatively little work has been done with fungi as a starting point, it was considered advisable to undertake a survey of the fungus flora of the different types of cane sugar and then to determine the power which the most important of these might have in causing deterioration. Subsequently the presence of these organisms in the sugar mill was studied with a view towards perfecting methods for the elimination of the dangerous invaders. It is not to be assumed that the experimental data and their interpretation set forth in the following pages are complete, but, rather, that they represent a report of progress which may prove of interest and possibly suggest methods for the conservation of that food product in which there is universal concern and which is continually subjected to deterioration by the activity of microorganisms.

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HISTORICAL.

Payen*[33] as early as 1851 noted a red coloration in sugar. Upon isolating the organism responsible he found that the spores were 1-2 microns in diameter. In a later communication he describes sugar which had irregular ridges on the crystals and cavities. On these were found filaments and their ramifications which were different from those of the previous organism described.

Shorey[40] found *Penicillium glaucum* in deteriorating Hawaiian sugars and regarded this organism as the factor responsible for inversion. This evidence, he claims, proves that the assertion "that all sugars which deteriorate do so because too moist or not alkaline enough" is unwarranted. The only place in the sugar-making process where the spores of fungi could gain access to the sugar is in the centrifugal and he finds a correlation between dusty districts and deteriorating sugars. Sterilization by dry steam in the centrifugal is recommended as protection against infection.

Kamerling[22], whose work is favorably discussed by Geerligs[17], found in dried sugar a fungus flora chiefly related to *Penicillium*. Over twenty varieties were observed and not less than nineteen belonged to this group. The author claims that during the course of deterioration of raw sugar the first attack is made by fungi.

Greig-Smith[20] advises the same procedure as Shorey[40] to guard against a bacterial infection. The former has isolated *Aspergillus glaucus* from small-grained, moist refined sugar, but has not considered its presence to be of as much significance as the potato group of bacilli, upon which he has worked so extensively.

Schöne[36 37 38] emphasized the importance of molds and torulæ in inversion and noted the presence of *Penicillia*, especially *P. glaucum* and *Mucor*. He found that considerable acidity was developed in sugar solutions inoculated with pure cultures of some of these organisms. In another connection he notes the isolation of *Penicillium* and *Rhizopus*.

Scott[39] observed the fungi on the surface of sugar liquors, noting *Penicillia* and *Aspergilli*. He found that sugars were attacked by these organisms in the following descending order: Low Brazilian, Peruvian, Jamaican and Javanese. The invert-

* The authors are indebted to Dr. C. A. Browne for this abstract.

ing power of the fungi was noted and precautions advised against infection.

Amons[2] isolated by plating on synthetic agar 4 *Aspergilli*, 2 *Penicillia* and a *Rhizopus*. Mycelial threads observed in moist sugars were identified as indicating the presence of *P. glaucum* in an active form. The deterioration of sugar by this fungus was demonstrated by inoculating sugar (sterilized with absolute alcohol) with spores and comparing the absorption of moisture (from a saturated sterile sugar solution under a bell jar) with a sterile check. It was found that the sterile sugar absorbed no moisture after thirty-three days, while the inoculated sugars absorbed a considerable amount of water. This is interpreted as signifying an appreciable fungus growth which inverted the sucrose, the hygroscopic reducing sugars being responsible for this absorption of moisture. It would have added to the value of this experiment had the chemical analyses for sucrose, reducing sugars, etc., been recorded at the beginning and end of the incubation period. Amons believes with Browne[7] and Noel Deerr that no single organism can be made universally responsible for the deterioration of sugar. Our evidence tends to corroborate this view.

Browne [6 7] in the course of his contributions to the sugar industry has included a great number of chemical observations concerning sugar deterioration. He has discussed the influence of temperature upon deterioration, deterioration without loss in polarization, the distribution of moisture, sweating, abnormal fermentations, the limit of deterioration, and has recorded practical deductions.

In his mycological observations Browne isolated and studied the inverting power of torulæ and two *Monilia* (named "nigra" and "fusca" respectively). The presence of fungi such as *Penicillia* were noted and their possible importance emphasized. Some interesting points are made with regard to sources of infection and consequent prevention of deterioration of raw cane sugars.

Owen [29 30 31 32] after an exhaustive survey of the activities of bacteria and torulæ investigated the role played by molds in sugar deterioration. From a large variety of Louisiana sugars

he isolated a number of *Aspergilli* and studied their activity in pure culture in sugar solutions of high concentration.

In an interesting group of experiments concerned with the conditions limiting the respective activities of the three groups of microorganisms in sugars, Owen[32] found that a fungus inoculation caused marked deterioration in a 69° Brix solution, which proved to be of too great a density for the activity of either bacteria or torulæ, the latter two groups inverting sucrose at 52° and 64° Brix respectively as the upper limits. He regards the fungi as constituting the most dangerous group of microorganisms in sugar because of their strong inverting power, their ability to exercise this power in highly concentrated solutions of sucrose of varying reaction, and also on account of their ability to develop on media which are very deficient in nutrients.

Amons[3] and Edson[13] have likewise indicated the activity of fungi in syrups.

Blake[4] has recently made a contribution to the deterioration of raw sugar, as has Prinsen Geerligs[18]. They are especially interesting in that they represent observations made from the factory rather than the laboratory standpoint, and will doubtless result in more enlightened practices because of the economic considerations involved.

PART I.

THE OCCURRENCE OF FUNGI IN CANE SUGAR.

It is evident from this review of previous investigations that there is an informing body of data which makes it imperative to subject the fungi to closer scrutiny with regard to their ability to deteriorate manufactured cane sugar.* With this end in view, a comprehensive survey of the fungus flora of cane sugar was undertaken. The samples under consideration included a variety of types of sugar from different sources and represented a considerable range in composition, age and keeping quality. For example, all sugars numbered up to 50 are samples which have been kept in tightly covered Mason jars for about two years. Numbers above 100 indicate sugars collected during the past year, many of these samples being employed in the laboratory within a very short time after manufacture, in some cases this being only a matter of a few hours. Samples of the principal types of sugar on the market are designated as follows: Plantation granulated, which is a dry white granulated sugar manufactured on a large scale in Louisiana and is sold without further refinement; "Plantation granulated undried," similar to the above but possessing more moisture; Yellow clarified, which is a still lower grade of the same process; Standard granulated, which is the dry refined sugar; Cuban raw, the standard raw sugar imported from Cuba; 96°, which takes its name from its polarization and implies a raw sugar; 96° washed, which means a low grade sugar "washed up to" a higher polarization; and, finally, Second, which is the term given to the raw sugar obtained from the extraction of first molasses.

The above mentioned types are representative not only of local but national sugar standards.

The isolation of fungi from these representative sugars was accomplished by the plating method commonly employed in bacteriological technique. In order to have a dilution water of as nearly the same approximate composition as the original source from which the isolations were being made, a 17° Brix solution made up with Plantation granulated sugar and sterilized in the

* Some of the results included in this bulletin have appeared in our preliminary communications [24] [25] [26].

autoclave at 15 pounds' pressure for 15 minutes was employed, thereby reducing any danger from plasmolysis, etc.[32] The method of plating was to dilute 100 gm. of each sugar in 100 cc. of dilution water, dissolve and shake thoroughly while removing 1 cc. with a sterile pipette to a sterilized Petri dish. Eight cc. of agar medium cooled to 35°-40°C. were then added and the plates incubated at 28°-30°C. for one week, counts being made after 3, 5 and 7 days respectively. With media having 50 per cent of sucrose the plates were examined again after 10 days. It may be observed, parenthetically, that we have found it desirable to pour media as cool as possible (even permitting a slight hardening in the bottom of the tube) to avoid any injurious heating of sensitive spores.

Media were employed which were considered to be adapted to a wide range of fungus development (sucrose being substituted for the sugar mentioned in the original formula), as well as some having a high sucrose content, and therefore more closely approximating the original habitat of the organisms isolated. All media were made up to a reaction of +1 by the usual titration method. As a rule, all media high in sucrose content were sterilized intermittently at streaming steam for 3 successive days, but towards the conclusion of the work a single sterilization in the autoclave at 15 pounds' pressure for 15 minutes was considered more satisfactory. This is in line with the results obtained by Mudge[28].

Under the high temperature and humidity conditions obtaining in our vicinity and because of the high concentration of the original product, it was desirable to use a stiff agar (20 gm. "Difco" per litre, all media being made in a double boiler) and it was found to be advantageous to plunge the media into cold water upon removal from the autoclave.

The synthetic fungi media in general use, Czapek's, Cook's II and Raisin agar, were compared with the media developed and recommended for sugar investigations by Amons[2], Browne[7] (using granulated sugar, instead of raw sugar, as suggested by this author) and Smith. In the latter case, Owen's modification of using 50 per cent sucrose was also employed. Hansen's yeast medium was included for comparison, as well as a modification of Czapek's agar by the authors. This modification con-

sisted of increasing the sucrose content from 30 to 50 gm. per litre and substituting 5 gm. of peptone and 1 gm. of ammonium nitrate for 2 gm. sodium nitrate in the original formula. This resulted in a more rapid colony development, which proved to be a time-saving device of some value. The composition of the media employed was as follows, using 20 gm. of agar in 1000 cc. of water:

Name	Sucrose, gm.	Peptone, gm.	K_2HPO_4, gm.	KCl, gm.	$MgSO_4$, gm.	$FeSO_4$, gm.	Additional gm.
Amons	100		1.0		0.5		2.0 KNO_3
Cook II	20	10	.25		.25		
Czapek	30		1.0	.5	.5	.01	2.0 $NaNO_3$
Hansen	100	10	3.0		2.0		
Kopeloff	50	5.0	1.0	.5	.25	.01	1.0 NH_4NO_3
Smith 10%	100	1.0		5.0			2.0 Na_3PO_4

The granulated sugar medium (identical with Browne's formula for raw sugar) was made by filtering a 30 per cent sucrose solution and diluting to 20° Brix. The Raisin agar was made by cooking 375 gm. of raisins for 1 hour at 80°C. and filtering, adding 2 gm. of NH_4Cl, and making up to one litre, with a reaction of +2.0. Smith's 50 per cent sucrose agar (Owen's modification) was identical with the formula given for the 10 per cent agar except that the sucrose content was increased from 100 to 500 gm.

Table I.

ISOLATION OF FUNGI FROM SUGAR.

Key:—Asp. f.—Aspergillus flavus. Asp. n.—Aspergillus niger.
Asp. nid.—Aspergillus nidulans. Asp. r.—Aspergillus repens.
Bl. Asp.—Blue Aspergillus. Clad.—Cladosporium.
Cit. I, II, III—Citromyces I, II, III.
Orange—Sterile Orange Fungus. Pen.—Penicillium sp.
Pen. div.—Penicillium divarticatum.
Pen. exp.—Penicillium expansum. Pen. lut.—Penicillium Luteum.
Pen. ros.—Penicillium roseum. Syn.—Syncephalastrum.
Trich.—Trichoderma. I, II, III, IV—Unknown Sterile I, II, III, IV.
F?—Unknown Fungus.

Type	No.	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
P. Gr.	1	0	Asp. n. Clad. Orange	Asp. n. Bl. Asp. Orange Cit. I Clad.	Clad.	0	Asp. n. II Bl. Asp.	Asp. n. Bl. Asp. Cit. I Orange	Asp. n.	Asp. n. Clad. II Bl. Asp.
96°	2	Asp. n. Bl. Asp.	Asp. n. II	Asp n. Bl Asp.	II	Asp. n II	Asp. n.	Asp. n.	O	Asp. n. Bl. Asp.
P. Gr.	3	Bl. Asp. Orange	Bl. Asp.	Asp. n. Bl. Asp. Orange II Clad.	Bl. Asp.	Asp. n. Bl. Asp.	Asp. n. Bl. Asp. III	Asp. n. Clad. Bl. Asp. Pen. lut.	Bl. Asp.	Asp. n. Bl. Asp. Pen. div.
96°W	7	Asp. n.	Asp. n.	Asp. n. Pen. div. IV Asp. fl.	Asp. n.	Asp. n. Bl. Asp.	Bl. Asp. II	Asp. n.	Asp. n.	Asp. n.
96°W	8	Asp. n. F?	Bl. Asp. Asp. fl.	Asp. n. Clad. Pen. div. II	O	Asp. n.	Bl. Asp.	Asp. n. Clad. Bl. Asp.	F?	
96°W	9	O	Bl. Asp.	Asp. n. Clad. Pen. exp. II	O	Asp. n. Bl. Asp.	Bl. Asp.	Bl. Asp. Clad.	Clad.	Bl. Asp. Asp. fl.

2nd	11	Asp. n. Bl. Asp. II	Asp. n.	Asp. n. Clad. II F?	F?	Asp. n. Orange III	Asp. n. III	Asp. n. Asp. fl.	Asp. n.	Asp. n.
96°W	12	Bl. Asp. F?	Asp. n. Bl. Asp.	Asp. n. Clad. IV Bl. Asp	O	Asp. n. Bl. Asp.	IV	Asp. n. Clad.	Clad. Pen. exp.	Bl. Asp. Clad. Cit. III
96°W	13	Clad. Bl. Asp.	Bl. Asp.	Asp. n. Bl. Asp. Clad. Pen. exp. Asp. fl.	O	Asp. n.	Asp. n. Asp. fl.	Asp. n.	Asp. n. Clad.	Asp. n. Clad.
96°W	14	Bl. Asp.	Bl. Asp.	Asp. n. Bl. Asp. Pen. exp.	O	Asp. n. Bl. Asp.	Bl. Asp.		Asp. n. Clad. Bl. Asp.	Bl. Asp.
P. Gr.	15	O	Asp. fl.	Asp. n II Bl. Asp.	O	Asp. n. Bl. Asp. IV Orange Cit. III	Asp. n. Bl. Asp. II III	Asp. n. Bl. Asp.	Clad F?	Asp. n Bl. Asp.
P. Gr.	16	Asp. n.	Asp. n. Clad. Bl. Asp. Orange III	Asp. n. Clad. Bl. Asp. II	Bl Asp.	II	Clad. III	Asp. n. Bl. Asp	Asp. n. Bl. Asp.	Asp. n. Bl. Asp.
P. Gr.	17	Bl. Asp. Orange	Bl. Asp. Orange	Asp. n. Bl. Asp.	Syn.	O	IV	Asp. n. Clad. Bl. Asp.	Bl. Asp. Syn.	Bl. Asp.
2nd	18	Clad.	Clad.	Clad. Asp. fl. II, IV.	O	Asp. n Bl. Asp.	O	Asp. n. Clad.	Asp. n.	
96°W	19	O	Bl. Asp.	Asp. n. Clad. Bl. Asp. IV	Clad.	Asp. n.	Asp. n. II	Asp. n.	Bl. Asp. Clad.	Asp. n.

Table I—Continued.

ISOLATION OF FUNGI FROM SUGAR.

Type	No.	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
2nd	20	Asp. n. Clad. Bl. Asp.	Asp. n. Syn. III	Asp. n. Clad. Bl. Asp. Pen. Lut. Orange III	Asp. n. Bl. Asp. IV	Asp. n.	Asp. n. Clad. Orange IV Cit. III	II Clad.	Asp. n. Bl. Asp. Clad. Pen. exp. Orange IV	Asp. n. Bl. Asp. Clad. II Trich.
96°	21	Asp. n. Clad. Bl. Asp. II	Asp. n. Orange	Asp. n. Clad. IV	Asp. n. Bl. Asp. IV Orange Asp. fl.	Asp. n.	Asp. n. Bl. Asp. Asp. fl.	Cit. I	Asp. n. Bl. Asp. Asp. fl.	Asp. n.
P. Gr. und.	22	Asp. n.	Clad. Bl. Asp. Orange	Asp. n. Clad. IV F?	Asp. n.	O	Asp. n. Bl. Asp.	Clad.	O	Asp. n. Asp. fl.
96°	23	Pen. exp. Syn.	Syn. Asp. n. Bl. Asp.	Asp. n. Bl. Asp. Clad. Syn.	Asp. n.	Asp. n.	Asp. n. Pen. exp.	Asp. n. Cit. I	O	Asp. n. Clad. Asp. fl.
2nd	24	Asp. n. Asp. fl.	Asp. n. III	Asp. n. Clad. Asp. fl. Orange Cit. III.	Asp. n. Clad.	Asp. n.	Asp. n. Bl. Asp. Asp. fl. III	Asp. n. Asp. fl.	Bl. Asp. III	Asp. n. Asp. fl.
P. Gr. und.	25	Asp. n.	Bl. Asp. Asp. fl.	Asp. n. Bl. Asp. Clad. II Asp. fl. Pen. exp.	O	II	Bl. Asp. Clad. II	Asp. n. Clad. Bl. Asp.	Bl. Asp. Pen. exp.	Bl. Asp.
96°	26	Asp. n.	Asp. n.	Asp. n. Pen. exp. Clad. II, IV	Asp. n. Bl. Asp.	Asp. n. Bl. Asp. III	Asp. n. Bl. Asp. Asp. fl.	Asp. n. Clad. Bl. Asp.	Bl. Asp.	Asp. n.

96°	27	O	Asp. n. Bl. Asp.	Asp. n. Bl. Asp. Clad.	Clad.	Asp. n.	Asp. n. Clad. Orange	Asp. n. Bl. Asp.	Clad.	Bl. Asp. III
P. Gr. und.	28	Bl. Asp.	Asp. n. Bl. Asp.	Asp. n. Clad. Pen. lut. Bl. Asp. II	Bl. Asp.	Asp. n.	Asp. n. Clad. Orange	Clad.	Bl. Asp.	Asp. n. Bl. Asp. II, III
P. Gr.	29	Bl. Asp.	Bl. Asp. Asp. fl.	Asp. n. Bl. Asp. Pen. exp. II	Clad.	Clad.	Bl. Asp.	Asp. n. Clad.	O	Clad. III
2nd	30	Asp. n. Bl. Asp. Clad.	Bl. Asp. Cit. III	Asp. n. Clad. II	Bl. Asp.	Asp. n. Clad.	Asp. n. Bl. Asp.	Asp. n.	Asp. n.	O
96°W	31	Asp. n. Clad. Bl. Asp. III	Clad.	Asp. n. Clad. Bl. Asp. Pen. exp. II	O	II	O	Asp. n.	Asp. n.	Asp. n. Clad.
P. Gr.	32	Asp. n.	Asp. n. II	Asp. n. Clad. Pen. exp. II	Asp. n.	Asp. n. Bl. Asp.	Asp. n. II	Asp. n. Pen. lut.	Asp. n. Bl. Asp. Clad.	Asp. n. Bl. Asp. Clad. Orange III
96°	33	Asp. n. II Pen. lut.	Asp. n. Bl. Asp. Clad. Pen. exp.	Asp. n. Bl. Asp. Pen. exp. II, III Orange	Asp. n. Asp. fl.	Asp. n. Clad. Syn. Orange	Asp. n. Bl. Asp. Clad. Asp. fl. Cit. III	O	Asp. n. Bl. Asp. Asp. fl. Orange III	Asp. n. III
96°W	34	Asp. n. Clad. II	Asp. n. Bl. Asp. II	Asp. n. Bl. Asp. Clad. Pen. exp.	II	O	Asp. n. Clad. Orange III	Asp. n.	Bl. Asp. Clad. Syn.	Asp. n
P. Gr.	35	Asp. n. Clad. Bl. Asp. III	Pen. exp. III	Asp. n. Pen. lut. Clad. Syn. Orange Bl. Asp.	O	O	Asp. n.	Asp. n. Cit. I	Bl. Asp.	Asp. n.

Table I—Continued.

ISOLATION OF FUNGI FROM SUGAR.

Type	No.	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
2nd	36	Bl. Asp.	Asp. n. Bl. Asp.	Asp. n. Asp. fl. Clad. Cit. I II	F?	O	Asp. n. Bl. Asp.	Asp. n. Bl. Asp.	F? IV	Asp. n. Bl. Asp. Clad. III
2nd	37	Asp. n. Bl. Asp.	Asp. n. Bl. Asp. Clad.	Asp. n. Bl. Asp. II Clad. Asp. fl. Orange		Asp. n.	Asp. n. Bl. Asp.	Asp. n.	Asp. n. Clad. Cit. III	Asp. n. Clad.
P. Gr.	39	Bl. Asp. Syn. Asp. n.	Bl. Asp. Syn.	Syn.	Clad.	O	Asp. n. Orange	Asp. n. Cit. I	Pen. exp. Clad.	Asp. n.
P. Gr.	41	Asp n. Bl. Asp. Syn.	Asp. n.	Asp. n. Clad. Bl. Asp. Asp. fl. II, III Syn.	O	O	Asp. n.	Bl. Asp. Clad. II	Bl. Asp. Syn.	Asp. n.
2nd	42	Asp. n.	Asp. n. Bl Asp.	Asp. n. Clad. II	O	Asp. n. Bl. Asp.	Asp. n. Bl. Asp.	Asp. n. Bl. Asp. Clad. Cit. I	Asp. n. Bl. Asp.	Asp. n.
P. Gr.	44	Bl. Asp. Syn.	Bl. Asp. Syn.	Asp. n. Bl. Asp. Syn. Clad.	Bl. Asp.		Asp. n.	Asp. n. Bl. Asp.	Bl. Asp. Syn.	Asp. n. Bl. Asp.
96°W	45	Bl. Asp.	Bl. Asp II	Asp. n. Bl. Asp. Pen. lut.	O	O	Asp. n. Clad. Bl. Asp. II	Asp. n.	Clad. Orange III	Asp. n. Clad.

Y. C.	50	Asp. n. Clad.	Asp. n. Bl. Asp. III	Asp. n.	Asp. n.		Asp. n. III Asp. fl.	Asp. n. Bl. Asp. IV	Asp. n. Clad.	Asp. n. Bl. Asp. Asp. fl. III
P. Gr.	100	Asp. n. Clad.		Asp. n.	O	Asp. n. Clad. Syn.	Syn.	Syn. Asp. n.	Asp. n. Bl. Asp. Syn. Orange	
St. G	101	Asp. n.		Asp. n. Bl. Asp. Orange	O	Asp. n. Clad. Bl. Asp. Orange	Asp. n. Orange	Asp. n. Orange	Clad.	
C. R.	102	Asp. n. Clad.		Asp. n. Bl. Asp.	Clad.	Asp. n. Bl. Asp. Clad.	Asp. n.	Asp. n. Clad.	Asp. n. Bl. Asp. Clad.	
P. Gr.	103	Asp. n. Bl. Asp. Clad. II		Asp. n Clad Bl. Asp. Orange IV		Asp. n. Bl. Asp.	Asp. n. Bl. Asp. Clad. IV Cit. III			Asp. n. Clad. Bl. Asp.
C. R.	104	Asp. n.		Asp. n. Clad. Orange Syn.		Asp. n. Bl. Asp. Syn.	Asp. n. Syn. Cit. III			Asp. n. Bl. Asp.
C. R.	105			Asp. n. Clad.		Asp. n.	Asp. n. Cit. III	Asp. n. Bl. Asp.	Asp. n. Asp fl. Bl. Asp Syn.	
C. R.	106			Asp. n.		O	O	Syn.	O	
C. R.	107			Bl. Asp.		Asp. n. Bl. Asp. Syn. Orange	Asp. n. Bl. Asp. Asp fl. Syn.	Asp. n. Bl. Asp. Orange	Asp. n. Bl. Asp.	
C. R.	108			Asp. n. Clad.		Syn.	O	O	Syn.	
C. R.	109			O		O	O	Syn.	Syn.	

Table I—Continued.

ISOLATION OF FUNGI FROM SUGAR.

Type	No.	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
C. R.	110			Asp. n. Clad. Bl. Asp. Orange		Asp. n.	Asp. n. Asp. fl. Orange	Asp. n.	Syn.	
C. R.	111			Orange		Orange IV	O	O	IV	
C. R.	112			Asp. n. Bl. Asp. Clad. Orange		O	Syn.	O	O	
C. R.	113			Asp. n. Bl. Asp.		Asp. n. Bl. Asp.	Asp. n. Bl. Asp.	Bl. Asp. Syn.	Asp. n. Clad. Bl. Asp. Syn. Asp. fl.	
P. Gr.	115	Asp. n. Clad. Bl. Asp. Cit. III III		Clad. Orange IV		Asp. n. Bl. Asp. Orange Clad.	Asp. n. Clad. Bl. Asp. Orange IV, III			Asp. n. Clad. Bl. Asp. Orange
P. Gr.	116	Asp. n. Clad. Bl. Asp.		Bl. Asp. Clad. IV Cit. III		Asp. n. Clad. Bl. Asp. IV, III	Asp. n. Syn. Clad. Bl. Asp.			Bl. Asp. Clad. III
P. Gr.	117	Asp. n. Clad. Bl. Asp.. II Syn.		Clad. Bl. Asp. Orange IV Cit. III		Asp. n. Clad. Bl. Asp. Syn.	Asp. n. Clad Orange Syn. III		Asp. n. Clad. Bl. Asp. Orange III	
P. Gr.	118	Clad. Bl. Asp. Orange Cit. III III		Bl. Asp. Clad. Orange Syn. IV		Asp. n. Orange Asp. fl.	Bl. Asp. Clad. Syn. Orange		Clad. Bl. Asp.	

P. Gr.	119	Asp. n. Clad. Bl. Asp.		Asp. n. Clad. Bl. Asp.		Asp. n. Clad. Bl. Asp.	Asp. n. Clad. Bl. Asp.			Asp. n. Bl. Asp.
Y. C.	120	Asp. n. Clad. Bl. Asp. III		Bl. Asp. Clad.		Asp. n. Bl. Asp. Asp. fl.	Asp. n. Clad. Bl. Asp. Orange III Syn.			Asp. n. Clad. Bl. Asp. III
Y. C.	123			Clad. Asp. n. Syn. Asp. f Asp. r. Bl. Asp.			Clad Asp. n. Asp. f. Syn. Bl. Asp. Pen. exp. Asp. r.			
Y. C.	124			Clad. Asp. r. Bl. Asp.			Clad. Bl. Asp. Pen. exp. Asp. n. Syn.			
Y. C.	125			Clad. Bl. Asp. Asp. f. Syn. Asp. n. Pen. exp. Asp. r.			Clad. Bl. Asp Asp. f. Syn. Asp. n. Pen. exp.			
Y. C	126			Clad. Bl. Asp. Pen. exp. Asp. n. III			Clad. Asp. f. Bl. Asp. Pen. exp. Asp. n. Pen ros.			
Y. C.	127			Clad. Asp. f. Bl. Asp. Pen. exp. Syn. Orange			Clad. Asp. f. Bl. Asp. Pen. exp. Orange			

Table I—Continued.

ISOLATION OF FUNGI FROM SUGAR.

Type	No.	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
Y. C.	128			Clad. Asp. f. Bl. Asp. Pen. exp. Syn. Asp. n.			Clad. Pen. exp. Bl. Asp. Syn.			
Y. C.	129			Clad. Bl. Asp.			O			
Y. C.	130			Clad. Asp. nid. Asp. r. Asp. n. Asp. f. Pen. exp.			Clad. Asp. f. Bl. Asp. Pen. exp. Asp. n. Cit. III			
Y. C.	131			Clad. Bl. Asp. Asp. n. Pen. div. Asp. sp.			Asp. f. Bl. Asp. Pen. exp. Asp. n. Pen. ros. Asp. sp.			
Y. C.	132			Clad. Asp. f. Bl. Asp. Pen. exp. Asp. nid. Asp n. Monilia n. III			Clad. Asp. f. Bl. Asp. Pen. exp. Monilia n. Asp. nid III			
Y. C.	133			Clad. Bl. Asp. Syn. Asp. n. Monilia n.			Clad. Bl. Asp. Asp. n. Syn.			

Y. C.	134			Clad. Asp. f. Bl. Asp. Asp. nid. Pen. exp. Asp. n. Asp. sp. Syn.			Clad. Asp. f. Pen. exp. Syn. Asp. nid. Asp. n. Pen. div. III			
Y. C.	135			Clad. Bl. Asp. Asp. nid. Asp. f. Asp. n. Asp. sp. Syn. III Pen. exp.			Clad. Asp. f. Pen. exp. Asp. nid. Pen. div. Asp. n.			
Y. C.	136			Bl. Asp. Syn.			Bl. Asp. Syn.			
Stand. G[1] Stand. G[2] Stand. G[3] Stand. G[4]				Asp. n. Bl. Asp. O Bl. Asp. Asp. n.			Clad. Syn. O Syn. Asp. n. Bl. Asp.			

The principal fungi isolated from the sugar samples on hand are indicated in Table I. These tabulated results represent the average of triplicate determinations. The summary of these data is recorded in Table II, which represents a calculation of these results in terms of per cent.

Table II.

PER CENT OF SAMPLES SHOWING FUNGUS.

Sugar Type	Total Number of Samples	Aspergillus niger	Cladosporium	Penicillium luteum	Citromyces I	Penicillium divart.	Unknown Sterile II	Unknown Sterile IV	Syncephalastrum	Sterile Orange	Citromyces III	Unknown Sterile III	Blue Aspergillus	Penicillium expansum	Aspergillus flavus
Plantation granulated.....	18	100	100	17	17	6	50	39	50	72	33	61	100	22	22
Plantation granulated undried..	3	100	100	33	0	0	67	33	0	67	0	33	100	33	67
Yellow clarified.....	16	81	94	0	0	19	6	0	63	13	6	38	100	70	75
Standard granulated.....	5	60	40	0	0	0	0	0	40	20	0	0	80	0	0
Cuban raw.....	11	82	64	0	0	0	0	9	73	45	18	0	64	0	36
96°.....	6	100	83	17	17	0	67	33	33	50	17	50	100	50	67
96° washed.....	10	100	90	10	0	20	70	30	10	20	10	30	100	60	40
2nd.....	8	100	100	12	25	0	75	37	12	50	50	50	100	12	60
Av.....	REL.	97	90	12	8	7	45	25	38	45	18	35	100	33	44

For example, the second column represents the total number of samples of each sugar type examined. If, as in the case of the Plantation granulated samples, *Aspergillus niger* was isolated in all 18 instances, the per cent of samples showing that fungus to be present would obviously be 100. But where this fungus was found in only 3 out of 5 samples of Standard granulated, the per cent recorded would be only 60. From this Table II it is possible to observe some interesting correlations. While it is difficult to ascribe a definite fungus flora to any particular type of sugar, yet it may be seen that the lower grades of sugar—i. e., "seconds" and 96° sugars—show a relatively high degree of infection qualitatively and quantitatively. While the results do not show the Plantation granulated undried to be more infected than dried Plantation granulated sugar, the greater number of samples of the latter presupposes an undue weighting and would lead to the inference that in reality the undried sugar has a relatively greater variety of infection. The fact that the Plantation granulated shows a greater infection than the Yellow clarified series is probably due to the greater age, and consequent exposure to sources of contamination. In accordance with the general expectation, the Standard granulated samples showed the least fungus infection.

The *Blue Aspergillus* and *Aspergillus niger* were isolated from practically every sample of sugar on hand, while the *Cladosporium* was found in 90 per cent of the cases. (The averages were computed on the basis of the *Blue Aspergillus* being rated at 100.) The *Sterile Orange fungus, Unknown Sterile II,* and *Aspergillus flavus* were isolated from about one-half the total number of samples, while the other organisms appeared less frequently in the following order: *Syncephalastrum, Unknown Sterile III, Penicillium exp., Unknown Sterile IV, Citromyces III, Penicillium luteum, Citromyces I, Penicillium divarticatum.* Those fungi which occurred in only rare instances were not tabulated, but are noted in Table III, which represents a list of the species isolated.

Table III.

FUNGI ISOLATED FROM CANE SUGAR.

SERIES I.

Aspergillus niger.
Aspergillus flavus.
Aspergillus nidulans.
Blue Aspergillus.
Citromyces *I, II, III.*
Penicillium expansum (?).
Penicillium divarticatum.
Penicillium luteum ser.
Syncephalastrum.
Trichoderma sp.
Sterile Orange fungus.
Unknown *I, II, III, IV.*

SERIES II.

Aspergillus sp.
Aspergillus, Bur. Chem. No. 3556.
Aspergilus repens.
Penicillium purpurogenum ser. near Penicillium pinophilum.
Penicillium purpurogenum ser near Penicillium luteum, a soil form.
Penicillium luteum—purpurogenum ser. near Penicillium regulosum.
Fusarium sp.
Monilia nigra (Browne).
Monascus purpurens ser.
Dematiaceous fungus.
Unknown *V, VI,* etc.
Penicillium roseum.

Series I comprises the group of fungi which appeared with greater frequency in the samples of sugar examined, while Series II includes the species appearing more infrequently. It will be noted that the species of *Penicillium* and *Aspergillus* are present in greatest number, an observation which is in agreement with that of previous investigators. It may be mentioned, incidentally, that *P. glaucum* is included under *P. expansum* (?) in the recent work of Dr. Thom*[42].

While there was no attempt made to record quantitative results, nevertheless the fact that these organisms appeared on media of abnormally high sucrose content adds evidence to their authenticity as true sugar fungi.

Since the plating method has the obvious limitation of not revealing whether the colony has developed from spores or mycelia, a microscopic examination was made of the samples of

* The authors gratefully acknowledge their indebtedness to Dr. Charles Thom and Miss M. Church, of the Bureau of Chemistry, U. S. Department of Agriculture, for these identifications.

sugar under investigation. In many instances mycelia were actually detected, but in the majority of cases the evidence was either negative or doubtful in character. The presence of a considerable amount of foreign organic material, such as strands from the bagging, plant tissues, etc., proved confusing. Mycelia were stained with Conn's[8] Rose Bengal by Dr. Thom and the authors in samples of Cuban raw sugar and inoculated sugars used in our later experimentation, thus proving conclusively the possibility of fungus development, multiplication and activity in such a highly concentrated substratum. (See Plates I and II.)

TABLE IV.

EFFICIENCY OF DIFFERENT MEDIA IN DEVELOPING VARIED TYPES OF FUNGI.

TYPE	Number	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
P. G.....	1	0	60	100	20	0	60	80	20	80
96°	2	100	100	100	50	50	50	50	0	100
P. G.....	3	40	20	100	20	40	60	80	20	60
96° W.....	7	25	25	100	25	50	50	25	25	25
96° W.....	8	50	50	100	0	25	25	75	25	..
96° W.....	9	0	25	100	0	50	25	50	25	50
2nd	11	75	25	100	25	75	50	50	25	25
96° W.....	12	50	50	100	0	50	25	50	50	75
96° W.....	13	40	20	100	0	20	40	20	40	40
96° W.....	14	33	33	100	0	67	33	..	100	33
P. G.....	15	0	20	60	0	100	80	40	40	40
P. G.....	16	20	100	80	20	20	40	40	40	40
P. G.....	17	67	67	67	33	0	33	100	67	33
2nd	18	25	25	100	0	50	0	50	25	..
96° W.....	19	0	25	100	25	25	50	25	50	25
2nd	20	50	50	100	50	17	85	34	100	85
96°	21	80	40	60	100	20	60	20	60	20
P. G. und.....	22	25	75	100	25	0	50	25	0	50
96°	23	50	75	100	25	25	50	50	0	75
2nd	24	40	40	100	40	20	80	40	40	40
P. G. und.....	25	17	34	100	0	17	50	50	34	17
96°	26	20	20	100	40	60	60	60	20	20
96° W.....	27	0	67	100	33	33	100	67	33	67
P. G. und.....	28	20	40	100	20	20	60	20	20	80
P. G.....	29	25	50	100	25	25	25	50	0	50
2nd	30	100	67	100	33	67	67	33	33	0
96° W.....	31	80	20	100	0	20	0	20	20	40
P. G.....	32	20	40	80	20	40	40	40	60	100
96°	33	50	67	100	33	67	83	0	83	33
96° W.....	34	75	75	100	25	0	100	25	75	25
P. G.....	35	67	33	100	0	0	17	33	17	17

TABLE IV—Continued.

TYPE	Number	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
2nd	36	20	40	100	20	0	40	40	40	60
2nd	37	33	50	100	..	17	33	17	50	33
P. G.	39	100	67	33	33	0	67	67	67	33
P. G.	41	44	15	100	0	0	15	44	30	15
2nd	42	25	50	75	0	50	50	100	50	25
P. G.	44	50	50	100	25	..	25	50	50	50
96° W.	45	25	50	75	0	0	100	25	75	50
Y. C.	50	50	75	25	25	..	75	75	50	100
St. G.	101	25	..	75	0	75	25	50	100	...
C. R.	102	67	..	67	33	100	33	67	100	...
P. G.	103	80	..	100	..	40	100	..	..	60
C. R.	104	25	..	100	..	75	75	..	..	50
C. R.	105	..	..	50	..	25	50	50	100	..
C. R.	106	..	..	100	..	0	0	100	0	..
C. R.	107	..	..	25	..	100	100	75	50	..
C. R.	108	..	..	100	..	50	0	0	50	..
C. R.	109	..	..	0	..	0	0	100	100	..
C. R.	110	..	..	100	..	25	75	25	25	..
C. R.	111	..	..	50	..	100	0	0	50	..
C. R.	112	..	..	100	..	0	25	0	0	..
C. R.	113	..	..	40	..	40	40	40	100	..
P. G.	115	83	..	50	..	67	100	..	..	67
P. G.	116	60	..	80	..	100	80	..	..	60
P. G.	117	100	..	100	..	80	100	..	100	..
P. G.	118	100	..	100	..	60	80	..	40	..
P. G.	119	100	..	100	..	100	100	..	..	67
Y. C.	120	67	..	33	..	50	100	..	..	67
Y. C.	123	..	..	85	..	..	100	..	..	..
Y. C.	124	..	..	60	..	..	100	..	..	..
Y. C.	125	..	..	100	..	..	65	..	..	..
Y. C.	126	..	..	85	..	..	100	..	..	..
Y. C.	127	..	..	80	..	..	100	..	..	..
Y. C.	128	..	..	100	..	..	67	..	..	..
Y. C.	129	..	..	100	..	..	0	..	..	..
Y. C.	130	..	..	100	..	..	100	..	..	..
Y. C.	131	..	..	85	..	..	100	..	..	..
Y. C.	132	..	..	100	..	..	87	..	..	..
Y. C.	133	..	..	100	..	..	80	..	..	..
Y. C.	134	..	..	100	..	..	100	..	..	..
Y. C.	135	..	..	100	..	..	67	..	..	..
Y. C.	136	..	..	100	..	..	100	..	..	..
Ave.	REL.	56	56	100	21	46	68	45	54	48

In Table IV is calculated the relative efficiency of the different media employed for yielding a variety of fungi. Thus, if from sample No. 1 (Type P. G.) the greatest number of different fungi isolated on any one medium was 5, and this occurred on Czapek's agar, the latter would receive a rating of 100. Since only 4 different organisms were isolated on Raisin agar, it received a rating of 80, and similarly with the other media on a purely qualitative basis. It will be seen from the general average that the largest variety of fungi was isolated on Czapek's agar, followed in order by Kopeloff (Modified Czapek), Amons, Cook II, Smith 10%, Smith 50%, Hansen, Raisin, and Granulated sugar. However, it is interesting to note that Kopeloff's Modification shows up to far better advantage on the newer samples No. 101-136. For this reason, as well as for the saving of time involved, we have used this agar for all plating in the laboratory when only one medium was being used. The rate of colony development was observed to be most rapid on this agar, a good growth being obtained after 3 days' incubation at 28°-30°C. Amons, Cook II, Czapek, Hansen and Smith 10% developed colonies in 4-7 days' time, while Smith 50% and Granulated sugar required 7-10 days. No definite correlation could be established between the media and organisms for which they were peculiarly adapted. The growth of fungi on Granulated sugar agar was particularly interesting because of the abnormally high sucrose concentration and slight amount of available nutrients.

PART II.

THE DETERIORATION OF CANE SUGAR BY FUNGI IN PURE CULTURE.

Having isolated the fungi which occur in cane sugar and found that they belonged chiefly to the *Aspergilli* and *Penicillia*, and having further established their presence in the mycelial as well as spore stage, it becomes necessary to further define the activities of these organisms. In the brief historical review presented in the introductory portion of this bulletin, mention has been made of the activities of fungi in inverting highly concentrated solutions of sucrose and even sugar itself. Effront[14] has an interesting discussion of the phenomenon based on the work of Duclaux[12], Raulin[35], Wasserzug[45] and others. Therefore, to determine whether or not the fungi isolated and named above were capable of deteriorating sugar, it was essential to carry out the work in pure culture on sterilized sugar. The problem of inoculation was indeed a difficult one because of the strict necessity for performing this operation without the introduction of any moisture. It was planned to add the mycelia and spores of each of the fungi to be studied to each of two widely divergent and representative types of sugar (Plantation granulated and Cuban raw) known to have somewhat above the normal amount of moisture. After incubating for a suitable period of time at an optimum temperature, this sugar was to be used as an inoculum in the succeeding experiments. The first question to arise, obviously, is whether the fungi could be recovered after inoculation. This is answered in the affirmative by the data recorded in Table V, which represent only the average of closely agreeing triplicate determinations.

TABLE V.

MYCOLOGICAL ANALYSIS OF SUGARS INOCULATED WITH FUNGI.
PLANTATION GRANULATED.

Number	Flask	Fungus	Presence		
1	300 cc.	Check	—	—	—
2	300 cc.	Aspergillus niger	+	+	+
6	200 cc.	Check	—	—	—
8	200 cc.	Aspergillus niger	+	+	+
11	200 cc.	Blue Aspergillus	+	+	+
14	200 cc.	Sterile Orange Fungus	+	+	—

TABLE V—Continued.

CUBAN RAW.

Number	Flasks	Fungus	Presence		
16	300 cc.	Check	—	—	—
18	300 cc.	Aspergillus niger	+	+	+
21	300 cc.	Blue Aspergillus	+	+	+
22	200 cc.	Check	—	—	—
24	200 cc.	Aspergillus niger	+	+	+
27	200 cc.	Blue Aspergillus	+	+	+

The method of procedure was as follows: 30 gm. and 50 gm. portions of each sugar were placed in 200 and 300 cc. cotton plugged Erlenmeyer flasks respectively. These sugars were sterilized in the Arnold for half an hour on each of 3 successive days and were shaken thoroughly at the end of each of the two 15-minute sterilization intervals, and finally stirred with a sterile spatula. After 3 days' incubation at 26°C. the contents of each flask were examined for sterility by plating on Kopeloff's agar.

Pure cultures of *Aspergillus niger, Blue Aspergillus,* and *Sterile Orange* fungus grown on Raisin agar were thoroughly shaken with 3-5 cc. of Raulin's solution, and 3 large platinum loopfuls of each added to the desired flask. The flasks were shaken thoroughly and incubated at room temperature for one week. At the end of this period they were plated on Kopeloff's agar. Thus in Table V it will be seen that there was a perfect recovery in every inoculated flask except one containing the *Sterile Orange* fungus, which was especially difficult to handle. The check flasks remained sterile.

With this as a starting point, a number of 250 gm. portions of Plantation granulated and Cuban raw sugars were placed in 1000 cc. Erlenmeyer flasks and sterilized in the Arnold for half an hour on 3 successive days. After 3 days' incubation at 28°C. they were sterilized again for 1 hour in the Arnold and incubated at room temperature until inoculated. This time no moisture was added, but mycelia and spores were scraped with a sterile platinum scoop from the surface of a large number of Petri dishes, in which the agar was covered with an abundant pure growth of the desired organism. The flasks were incubated at

room temperature and shaken thoroughly every day for 3 weeks. Counts were then made on Kopeloff's agar and the results recorded in Table VI.

TABLE VI.

NUMBER OF ORGANISMS IN INOCULATED SUGARS.

PLANTATION GRANULATED.

Number	Fungus	1-1000 Dilution Colonies per Plate		Av. Number per 1 gm.	1-8000 Dilution		Av. Number per 1 gm.
1	Aspergillus niger...	50	47	49,000	..	..	.....
2	Sterile Orange.....	0	0	1,000	..	..	.....
3	Cladosporium	10	16	13,000	..	..	.....
4	Penicillium No. 2694	250	250	250,000	30	33	256,000
5	Blue Aspergillus....	175	192	184,000	20	21	168,000
6	Aspergillus flavus...	15	13	14,000	2	1	12,000
7	Syncephalastrum ...	0	0	1,000	..	..	.....
8	Check	0	0	....	..	..	.....
	CUBAN RAW.						
10	Aspergillus niger...	170	150	160,000	..	..	.....
11	Sterile Orange.....	0	0	1,000	..	..	.....
12	Cladosporium	0	0	1,000	..	..	.....
13	Penicillium No. 2694	22	20	21,000	..	..	.....
14	Blue Aspergillus...	325	500	413,000	78	84	648,000
15	Aspergillus flavus...	90	85	88,000	..	..	.....
16	Syncephalastrum ...	0	0	1,000	..	..	.....
17	Check.	0	0	....	..	..	.....

It may be mentioned, parenthetically, that it is always our custom to incubate dilution flasks with the plates for comparison as to growth, as the results prove of interest.*

It will be seen that the average number of organisms per gm. of inoculum varied from 1,000 to 648,000, and usually 10 gm. of each inoculum were added to 150 gm. of sterile sugar, which proved to be satisfactory.

In conducting an experiment involving the inoculation of sterile sugar with pure cultures of fungi, it was of interest to note that the prevailing conception among mycologists with regard to the limit of concentration for the growth of *Penicillia*

* In this connection, it is a pleasure to thank Mr. W. L. Owen, who has volunteered many valuable suggestions.

and *Aspergilli* has generally been considered to be between 60-70 per cent. The work of Eschenhagen[15], Raciborski[34] and others may be cited in this connection. As Thiele[41] has pointed out, the temperature boundaries of these organisms depend upon the nutrients present, as well as the varying concentration and reaction of the medium. It was established by this investigator that with increasing concentrations the temperature limits are increased. Thus where the optimum temperature for *Penicillium* is generally about 25°C. where a 50 per cent sugar solution was employed the organism grew well at 34°C. A similar 10 per cent increase is obtained with *Aspergillus niger*. Duclaux[12] and Wasserzug[45] have shown that *Aspergillus niger, Penicillium glaucum* and many other fungi are capable of inverting sucrose. The former claims that 35°C. is the optimum temperature for *Aspergillus niger,* which corroborates Raulin's[35] limits of 19-43°C. for this organism. Moreover, the latter regards humidity as directly affecting the growth of the fungus, as well as the influence of various nutrients. In the light of such considerations it was planned to incubate all inoculated flasks at 30°C. The sugars employed in this experiment designed to study the deteriorative activity of pure cultures of fungi isolated from sugar were of the three principal types, namely: Plantation granulated, Refined and Cuban raw. One hundred and fifty gm. portions were weighed into sterilized 500 cc. Erlenmeyer flasks and sterilized as usual and examined for sterility. To each of three flasks of each type of sugar 10 gm. of inoculum containing *Aspergillus flavus, Aspergillus niger, Blue Aspergillus, Syncephalastrum,* and sterile sugar alone, respectively, were added. An inoculum of Cuban raw sugar was used for the Cuban raw series, while Plantation granulated was used for both the Plantation granulated and the Refined series. The inoculated flasks were incubated at 30°C. for 4 months, with no provision for replacing any of the moisture which evaporated. Under such circumstances the sugars dried out to such an extent as to make the moisture content negligible. At the end of the incubation period, the contents of each flask were analyzed and the results recorded in Tables VII-IX.

Before discussing these data, however, it is well to bear in mind the general concensus of opinion, both in the laboratory

and the field, to the effect that the "Factor of Safety" is a fairly reliable criterion for the keeping quality of a sugar. In other words, it is generally conceded that $\frac{\text{Moisture}}{100 - \text{Polarization}}$ should yield a fraction lower than .33 to guarantee the non-deterioration of an unwashed raw sugar. Browne[7] more recently has shown that for Cuban and Porto Rican sugars this constant is a little too high and the value 0.3 is more nearly correct. However, for washed 96-test sugars this factor becomes of less value. Furthermore, according to Owen[32], it is inapplicable to white sugars "because in such cases it is the total moisture that must be considered, since the density of the film can not exceed the maximum concentration in which microorganisms can destroy sucrose."

TABLE VII.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

PLANTATION GRANULATED.

Number	Fungus	*Presence after 4 months	S. Pol.	Clerget	Reducing Sugars	Moisture	Moisture ratio
7	Check	—	99.2	99.26	0.16	0	
10	Check	—	99.0	98.88	.16	0	
14	Check	—	98.9	98.73	.15	.07	
AVE.		—	99.0	98.96	.16	.02	0.02
12	Aspergillus flavus. . . .	—	99.1	99.06	.16	.02	
13	Aspergillus flavus. . . .	—	98.9	98.88	.16	0	
AVE.		—	99.0	98.97	.16	.01	0.01
16	Blue Aspergillus.	+	98.9	98.27	.17	.11	
17	Blue Aspergillus.	+	98.6	98.50	.16	.02	
18	Blue Aspergillus.	—	99.2	99.12	.15	.07	
20	Blue Aspergillus.	—	99.1	99.22	.16	.12	
AVE.		±	98.9	98.78	.16	.08	0.07
15	Syncephalastrum	—	99.0	98.88	.13	.08	
21	Syncephalastrum	+	98.9	98.66	.14	.05	
23	Syncephalastrum	—	98.9	98.94	.15	.08	
25	Syncephalastrum	—	98.9	98.59	.15	.05	
AVE.		∓	98.9	98.77	.14	.07	0.06
4	Aspergillus niger. . . .	—	99.0	99.06	.18	0	
8	Aspergillus niger. . . .	—	99.4	99.25	.15	0	
28	Aspergillus niger. . . .	+	98.9	98.59	.13	.10	
29	Aspergillus niger. . . .	+	98.8	98.43	.15	.04	
AVE.		±	99.0	98.84	.15	.03	0.03

* Average of triplicate plates with two dilutions.

The column marked "Moisture" in Table VII indicates the presence of a very low moisture content and the "factor of safety" or "moisture ratio" in this series would be considerably below 0.10. Therefore, marked deterioration would not be anticipated. That such is actually the case may be seen in the single polarization (S. Pol.) sucrose Clerget (Clerget) and reducing sugars values set forth. Practically all these determinations are within the limit of experimental error. Slight indications of the loss of sucrose are perhaps to be noted in the case of the *Blue Aspergillus* (flasks 16 and 17) and *Aspergillus niger* (flasks 28 and 29). The same general negative evidence is to be gathered from Table VIII.

TABLE VIII.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

REFINED.

Number	Fungus	Presence after 4 months	S. Pol.	Clerget	Reducing Sugars	Moisture	Moisture ratio
2	Check	—	99.4	99.72	.09	.04	
3	Check	—	99.7	99.84	.05	.02	
5	Check	—	99.4	99.38	.05	.02	
6	Check	—	99.4	99.49	.04	.03	
AVE.			99.5	99.61	.06	.03	0.06
7	Aspergillus flavus....	+	99.3	99.33	.05	.03	
8	Aspergillus flavus....	+	99.5	99.76	.05	0	
AVE.		+	99.4	99.54	.05	.02	0.03
14	Blue Aspergillus.....	+	99.5	99.90	.04	.03	
15	Blue Aspergillus.....	+	99.5	99.46	.04	.05	
16	Blue Aspergillus.....	+	99.3	99.26	.04	.04	
17	Blue Aspergillus.....	+	99.3	99.16	.04	.04	
AVE.		+	99.4	99.44	.04	.04	0.07
18	Syncephalastrum	+	99.6	99.61	.04	0	
19	Syncephalastrum	—	99.7	99.80	.04	0	
21	Syncephalastrum	+	99.2	99.04	.04	0	
23	Syncephalastrum	+	99.3	99.30	.06	0	
AVE.		+	99.4	99.44	.05	0	0
26	Aspergillus niger....	+	99.7	99.72	.04	0	
27	Aspergillus niger....	+	99.7	99.80	.04	0	
28	Aspergillus niger....	—	99.7	99.91	.05	0	
29	Aspergillus niger....	+	99.6	99.65	.05	0	
AVE.		+	99.7	99.77	.05	0	0

Only the slightest signs of inversion are exhibited by *Blue Aspergillus* (16 and 17) and *Syncephalastrum* (21 and 23).

TABLE IX.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

CUBAN RAW.

Number	Fungus	Presence after 4 months	S. Pol.	Clerget	Reducing Sugars	Moisture	Moisture ratio
1	Check	—	95.7	96.27	1.04	0.83	
2	Check	—	95.7	95.99	1.09	1.06	
4	Check	—	95.6	95.97	1.04	1.15	
AVE.		—	95.7	96.08	1.06	1.01	0.26
17	Aspergillus flavus. . .	—	95.6	95.93	1.09	1.07	
18	Aspergillus flavus. . .	—	95.6	95.78	1.09	1.06	
AVE.		—	95.6	95.86	1.09	1.07	0.24
6	Blue Aspergillus. . . .	+	95.0	95.51	1.13	0.96	
7	Blue Aspergillus. . . .	—	95.0	95.51	1.04	1.00	
8	Blue Aspergillus. . . .	+	95.1	95.54	1.56	1.14	
10	Blue Aspergillus. . . .	—	95.2	95.74	1.19	0.94	
AVE.		+	95.1	95.58	1.23	1.01	0.20
12	Syncephalastrum . . .	+	95.7	96.27	1.09	1.11	
13	Syncephalastrum . . .	+	94.8	95.28	1.09	1.06	
15	Syncephalastrum . . .	+	95.4	95.86	1.09	1.10	
16	Syncephalastrum . . .	+	95.6	96.02	1.09	0.99	
AVE.		+	95.4	95.86	1.09	1.06	0.23
26	Aspergillus niger. . . .	+	95.0	95.51	1.11	1.05	
27	Aspergillus niger. . . .	—	95.6	96.01	1.11	1.23	
28	Aspergillus niger. . . .	—	95.5	95.93	1.11	1.22	
29	Aspergillus niger. . . .	+	95.6	96.01	1.14	1.29	
AVE.		—	95.4	95.86	1.12	1.20	0.26

However, in the Cuban raw series shown in Table IX, where the moisture content is appreciable, though well below the factor of safety limit, some evidence of the activity in inverting sucrose may be found in the case of the *Blue Aspergillus* and to a lesser degree in *Aspergillus niger*. This experiment consequently may be regarded as establishing the lower limit of the deterioration of manufactured cane sugar by the fungi employed. While a factor of safety of 0.1-.20 is practically a guarantee of non-deterioration in the above sugars, nevertheless, even at this ab-

normally high concentration there appears to be some slight evidence of possible activity on the part of these organisms. The bearing which this has upon the general factor of safety rule will be dealt with in more detail in a subsequent experiment in Part II.

All the sugars were plated in triplicate on Kopeloff's agar, using 1 cc. of dilution (20 gm. sugar to 40 cc. 17° Brix sugar solution) to 8 cc., and the presence or absence of the inoculated fungi indicated by plus and minus signs, respectively, in the third column of the foregoing tables. There is little consistency with regard to the recovery of these fungi and there is, furthermore, no indication of any multiplication of the organisms involved. A microscopical examination failed to establish the presence of mycelia in any of these sugars.

TABLE X.

ANALYSES OF SUGAR INOCULATED WITH DIFFERENT FUNGI.

PLANTATION GRANULATED.

Number	Fungus	Presence after 4 months	Single Pol.	Clerget	Reducing Sugars	Moisture	Moisture ratio	*Dry Basis* S. P.	*Dry Basis* S. C.
103	Check	—	99.6	99.58	0.14	0.09	.22	99.7	99.7
20	Cladosporium	+	98.7	98.86	0.25	0.19	.15	98.8	99.0
21	Aspergillus flavus. .	+	99.0	98.73	.15	.06	.06	99.0	98.8
22	Blue Aspergillus. . .	+	98.5	98.54	.44	.19	.13	98.6	98.7
23	Penicillium 2694. . .	+	98.9	98.82	.20	.22	.20	99.1	99.0
24	Syncephalastrum. . .	+	99.3	99.23	.14	.06	.09	99.4	99.3
24A	Unknown I.	—	99.3	99.20	.17	.08	.10	99.1	99.3
25	Trichoderma	+	98.7	98.65	.31	.20	.15	98.9	98.8
26	Citromyces	+	98.7	98.75	.14	.03	.02	98.7	98.8
27	Asp. nidulans.	+	98.8	98.87	.15	.09	.07	98.9	98.9
28	Citromyces II.	+	99.2	99.07	.14	.07	.10	99.3	99.1
29	Pen. pinophilum. . .	—	98.8	98.83	.28	.23	.20	99.0	99.0
30	Citromyces III. . . .	—	99.0	99.22	.15	.11	.17	99.1	99.3
31	Penicillium div. . . .	+	99.5	99.54	.19	.09	.06	99.6	99.6
32	Unknown II.	+	98.4	98.29	.14	.11	.06	98.5	98.4
33	Sterile Orange. . . .	—	99.1	98.76	.16	.07	.07	99.2	98.8
34	Unknown III.	+	98.9	98.92	.23	.15	.13	98.9	99.1
36	Aspergillus niger. . .	+	99.5	99.50	.15	.06	.04	99.6	99.6
37	Unknown IV.	+	97.4	97.33	.31	.31	.12	97.7	97.7
38	Unknown V.	—	98.9	98.77	.31	.30	.27	99.2	99.1

In Table X are presented the analyses of the sugars inoculated with the various fungi isolated and which were to be used whenever required for inoculation. In this Plantation granulated series the moisture ratio was uniformly low and the amount of deterioration, in consequence, was relatively slight, as will be seen from the values of sucrose Clerget on the dry basis (which represent the most accurate criteria for comparison). Some of the *Unknown Sterile fungi,* notably II, and the *Blue Aspergillus* caused an appreciable inversion. It will be noted, furthermore, that the *Blue Aspergillus* is responsible for the greatest amount of reducing sugars throughout the series.

TABLE XI.

ANALYSES OF SUGAR INOCULATED WITH DIFFERENT FUNGI.

CUBAN RAW.

Number	Fungus	Presence after 4 months	Single Pol.	Clerget	Reducing Sugars	Moisture	Moisture ratio	Dry Basis	
								S. P.	S. C.
104	Check	—	96.2	96.24	1.00	1.00	.26	97.2	97.2
20	Cladosporium	+	95.9	95.74	1.09	1.07	.26	97.0	96.8
21	Aspergillus flavus..	+	96.0	95.93	1.09	1.26	.31	97.2	97.1
22	Blue Aspergillus...	+	92.3	91.07	2.28	2.28	.30	94.5	93.2
23	Penicillium 2694...	+	96.2	96.11	0.98	1.04	.27	97.2	97.1
24	Syncephalastrum ..	+	95.9	95.81	1.09	1.18	.29	97.0	96.9
24A	Unknown I.....	—	96.2	96.11	1.09	1.33	.35	97.5	97.4
25	Trichoderma	+	96.2	96.11	1.09	1.35	.35	97.5	97.4
26	Citromyces I.....	+	95.5	95.44	1.11	1.55	.34	97.0	96.9
27	Asp. nidulans.....	+	96.2	96.13	1.09	1.26	.33	97.4	97.3
28	Citromyces II.....	—	96.1	96.05	1.14	1.23	.32	97.3	97.2
29	Pen. pinophilum...	—	96.2	96.13	1.09	1.26	.33	97.4	97.3
30	Citromyces III.....	—	96.0	95.89	1.09	1.16	.29	97.1	97.0
31	Penicillium div....	+	95.9	95.75	1.09	1.00	.24	96.9	96.7
32	Unknown II.....	+	96.0	95.75	1.09	1.30	.32	97.2	97.0
33	Sterile Orange.....	—	95.9	95.75	*	1.43	.35	97.3	97.1
34	Unknown III.....	+	94.9	95.06	1.11	1.44	.28	96.3	96.4
36	Aspergillus Niger...	+	96.1	96.05	1.09	1.33	.34	97.4	97.3
37	Unknown IV.....	+	96.0	96.01	1.11	1.29	.32	97.3	97.3
38	Unknown V.....	+	95.6	95.74	1.56	1.68	.38	97.2	97.4

* Lost.

In the same way, in the Cuban raw series shown in Table XI, where the moisture ratio was considerably higher than in the preceding series, a considerable inversion as indicated by the

low Clerget value was obtained with the *Blue Aspergillus*. Correspondingly, the greatest value for reducing sugars was noted for the same organism. In a similar manner a number of other fungi were more active in the Cuban raw than in the Plantation granulated series, accentuating again the importance of moisture as a limiting factor in the deterioration of sugar by micro-organisms. The results obtained with these pure cultures substantiate the more general conclusions arrived at by Browne[7] and Owen[32] that deterioration in raw sugar takes place when the moisture factor is between 0.25 and 0.30. In fact, there are ample grounds for regarding the activity of fungi as proceeding at higher concentrations than any of the other groups of micro-organisms previously studied, and later experiments will show that the factor of safety rule is not reliable where there is a considerable infection due to the presence of fungi. The mycological observations carried out by the plating method previously described revealed the presence of fungi in a larger number of instances than in the preceding experiment (Tables VII-IX), where the moisture content was decidedly lower.

Having established the lower limit for the deterioration of sugar by the fungi isolated and witnessed the stimulation in activity of these organisms where the moisture content was increased, it became desirable to approximate more nearly the optimum conditions for the full exercise of their power in this direction. The following experiment was conducted in a manner similar to that previously described, with one paramount difference, namely, the sterilized sugars (of the three principal types) were permitted to absorb moisture in the autoclave before inoculation. This was accomplished in the following manner. As previously, 150 gm. portions of Plantation granulated, Refined and Cuban raw sugars were placed in 500 cc. sterilized Erlenmeyer flasks and sterilized in the usual manner, with frequent shaking to prevent caking. It was observed that if the sugar was shaken by tapping the flask against the hand from the time it was removed from the Arnold until it was thoroughly cool, there would rarely be any lumps formed. Only flasks free from fungus contamination and having the slightest bacterial content were used. The flasks were then placed in the autoclave, and the cotton plugs removed. Several small pans of boiling water

were placed inside, as well as wads of cotton soaked in boiling water. The autoclave was heated sufficiently to permit the sugars to become slightly warmed and they remained in the "humidor" for 4 hours. From time to time the water in the pans was brought to the boiling point and the autoclave heated slightly. The sugars were analyzed for moisture and the process continued until the desired moisture ratio was obtained with each sugar. The initial moisture ratio of these sugars averaged about 0.45.

The method previously described for preparing an inoculum was improved upon in the following manner. Pure cultures of each fungus to be employed were grown on numerous plates of Kopeloff's agar several weeks before use, to obtain an abundant spore production. Large samples of Plantation granulated and Cuban raw sugar were sterilized in the usual way. Then in the sterile chamber (used only for careful bacteriological work) successive portions of sugar from a single flask would be introduced into the Petri dishes containing the desired organism, shaken slightly and returned to the flask through a sterile aluminum funnel. In this way the whole mass of sugar in the flask received a liberal coating of spores and thorough shaking resulted in a uniform distribution. These flasks containing the inoculum were incubated together with uninoculated sugar (to be added to the check flasks) at 30°C. for 2 weeks and shaken daily. Ten gm. portions of inoculum were used in inoculating the sterile sugars previously described. Before inoculation, however, a count of spores in each inoculum was made by making a 1 to 2 dilution and examining with a Zeiss blood counting cell as used by us[23] in another connection. These data are to be found in Tables XV-XVII.

All flasks containing sterilized sugars made up to optimum moisture content were inoculated in triplicate with 10 gm. portions of the inoculum containing the desired organism (uninoculated portions of the same sugar being used for the check flasks) and incubated at 30°C. for one month. The humidity of the incubator was maintained at 65-75 per cent. At the end of the incubation period, which was relatively short, the contents of all flasks were analyzed and the averages of closely agreeing triplicate determinations recorded in Tables XII-XIV.

TABLE XII.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

PLANTATION GRANULATED.

FUNGUS	Mycelia	S. P.	S. Clerget	Reducing Sugars	Moisture	Moisture ratio	*Dry Basis* S. P.	*Dry Basis* S. C.
Check	—	98.6	98.3	0.23	.44	.31	98.9	98.8
Aspergillus flavus	—	98.5	98.3	0.26	.47	.27	98.9	98.7
Blue Aspergillus	+	97.1	97.2	0.69	.88	.31	97.9	98.0
Syncephalastrum	—	98.6	98.5	0.24	.37	.26	98.9	98.9
Aspergillus niger	+	97.4	97.4	0.44	.65	.27	98.1	97.9

It will be readily observed in Table XII that the *Blue Aspergillus* and *Aspergillus niger* are responsible for considerable inversion in Plantation granulated sugar, as represented by the reduction in Clerget values on the dry basis when compared with the check. There is a deterioration of almost 1% in this short period. That this is a true inversion is corroborated by the tripling and doubling, respectively, in the amount of reducing sugars. The moisture ratio in this series approaches the critical value arrived at in the valuable contributions of Browne[7], Owen[32] and others. There is, consequently, no doubt that both the *Blue Aspergillus* and *Aspergillus niger* at moisture ratios of 0.31 and 0.27 respectively are capable of inducing a serious loss in Plantation granulated sugar. Two of the other organisms, *Aspergillus flavus* and *Syncephalastrum,* on the other hand, exhibit little if any tendency to cause inversion, even under these circumstances. It is especially noteworthy in this connection to emphasize the fact that the two fungi capable of producing marked deterioration are the same which appeared most frequently in the large number of various sugar samples examined and discussed in Part I of this bulletin, namely, the *Blue Aspergillus* and *Aspergillus niger.*

Table XIII.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

REFINED.

Fungus	Mycelia	S. P.	S. Clerget	Reducing Sugars	Moisture	Moisture ratio	Dry Basis	
							S. P.	S. C.
Check	—	99.6	99.4	.11	.16	.35	99.7	99.6
Aspergillus flavus	+	98.2	98.2	.24	.42	.23	98.6	98.6
Blue Aspergillus	+	97.9	97.9	.57	.63	.30	98.6	98.3
Syncephalastrum	—	99.4	99.4	.22	.19	.34	99.6	99.6
Aspergillus niger	—	98.8	98.8	.41	.51	.41	99.3	99.3

The Refined series recorded in Table XIII yields much the same evidence. Here, again, where the moisture ratio is in the vicinity of 0.3 and deterioration is to be expected, the *Blue Aspergillus* and the *Aspergillus niger* are active in inversion, as evidenced by the low Clerget and high reducing sugar values. *Aspergillus flavus* also appears to have some activity in the present series. This fungus caused a loss of 1 per cent of sucrose, while the *Blue Aspergillus* was responsible for a still greater inversion. *Aspergillus flavus* produced this striking deterioration at a moisture ratio of only 0.23, while the *Blue Aspergillus* operated at 0.30.

Table XIV.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

CUBAN RAW.

Fungus	Mycelia	S. P.	S. Clerget	Reducing Sugars	Moisture	Moisture ratio	*Dry Basis*	
							S. P.	S. C.
Check	—	95.1	95.2	0.97	1.98	.40	97.0	97.2
Aspergillus flavus	—	95.2	95.2	1.05	1.90	.40	97.0	97.1
Blue Aspergillus	—	92.7	93.4	2.01	2.50	.34	95.0	95.6
Syncephalastrum	—	95.0	94.9	1.01	2.03	.40	96.5	96.8
Aspergillus niger	—	94.6	94.8	1.09	2.01	.37	96.5	96.7

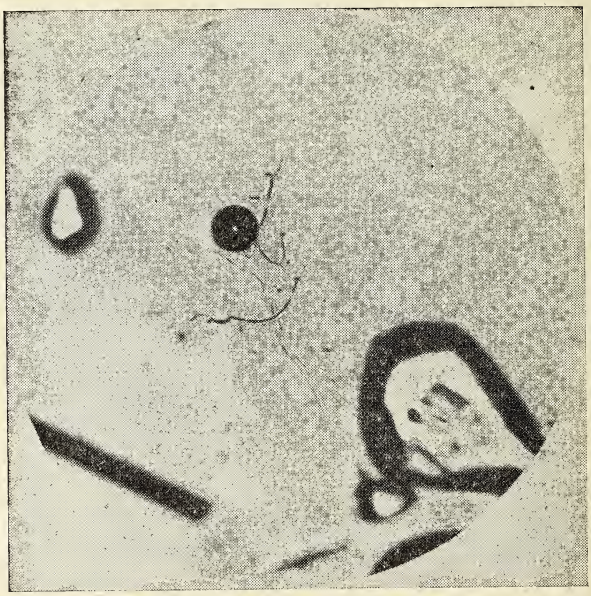


Plate I.

Mycelial threads of *Blue Aspergillus* in center of the field adjoining crystals of Plantation granulated sugar, from inoculated flask, after one month's incubation.

Photomicrograph enlarged four times from original taken with 16 mm. objective.

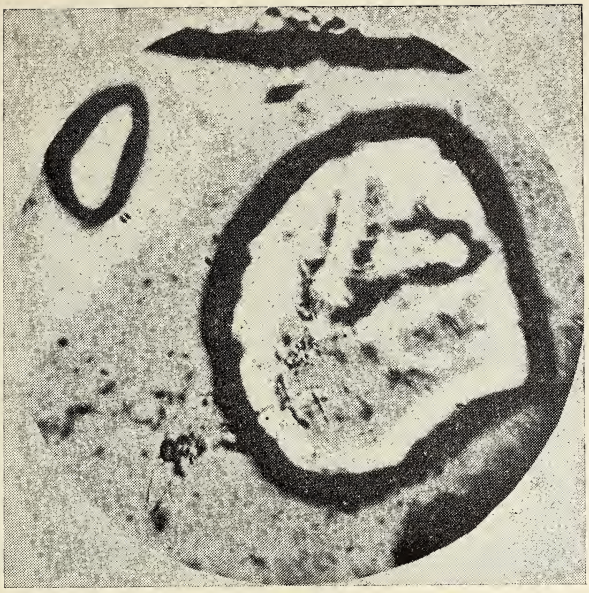


PLATE II.

Mycelial threads on and near large crystals of Cuban raw sugar. Uninoculated sample, collected on arrival from Cuba.

Photomicrograph enlarged four times from original taken with 16 mm. objective.

In the Cuban raw series in Table XIV the results parallel those previously noted. Again the *Blue Aspergillus* and *Aspergillus niger* are very active, the former causing a loss of 1.6 per cent sucrose Clerget on the dry basis and proving this to be a true case of inversion by a doubling in the amount of reducing sugars. *Syncephalastrum* exhibits some inclination to invert the sucrose present. In this series the moisture ratio was such as to warrant the sugar "unsafe" and it is patent that the fungi in question are capable of taking advantage of optimum environmental conditions.

In comparing the activity of the *Blue Aspergillus* throughout the three types of sugar it will be seen that the maximum loss of sucrose occurred in the Cuban raw sugar, and that this coincides with the greatest moisture ratio, 0.34. In fact, the individual instance of greatest loss of sucrose throughout the experiment is to be found in flask 9 of this series, which shows a loss of 2.4 per

cent in the Clerget on the dry basis and a correspondingly high value for reducing sugars. The Refined series showed a loss of 1.3 per cent Clerget on the dry basis, with a moisture ratio of 0.30. It is altogether probable that the deterioration was greater in this than in the Plantation granulated series (which had practically the same moisture ratio and caused a loss of only 0.8 per cent) because of the lower density of the hygroscopic film about each sugar particle. This involves a discussion of the validity of the factor of safety for washed sugars.

It has been previously pointed out that the reliability of this factor decreases with the artificial dilution of the hygroscopic film induced by washing the sugar. The polarization of sugar is increased by washing, which means a proportional increase in purity, hence a removal of solids non-sucrose from the film. This implies a film of lower density and consequently results in a greater tendency towards deterioration. Browne[7] states the matter succinctly in a discussion of deductions from the "factor of safety" rule. He claims that "if a fixed ratio between moisture and non-sucrose is the governing factor in the keeping quality of raw cane sugars, there are a number of deductions or corollaries which must follow from such a proposition.

The first corollary which we will consider is that slight fluctuations in moisture content have a much greater influence upon the keeping quality of high grade than of low grade sugars. Thus 0.1 per cent increase in moisture will raise the factor of a 99° sugar* with 0.28 per cent moisture from 0.28 to 0.35, but will raise the factor of a 90° sugar of 2.80 per cent moisture from 0.28 to only 0.29. In other words, a high-grade sugar of good keeping quality can be made unfit for storage by the absorption of only 0.1 per cent moisture, while the keeping quality of a low-grade sugar having the same factor will not be sensibly affected. This conclusion is abundantly confirmed not only by laboratory tests but by practical experience. The storage of high-grade raw sugars or of moist refined sugars has always been regarded as hazardous. *Even white granulated sugar has been found to deteriorate in a humid atmosphere, owing to the absorption of moisture.*†

* Typographical error corrected in correspondence.
†The italics are ours.

This is precisely the condition we achieved in this experiment through the activity of the fungi in pure culture.

The significant feature of the factor of safety rule, from the biological standpoint, is that it attempts to define the limit of concentration beyond which microorganisms cannot operate, and consequently sucrose is not destroyed by inversion. The data which were presented in Tables VII-XIV, however, when considered as a whole, make it evident that the factor of safety for white sugars is very close to 0.1, while for Cuban raw sugar it is nearer 0.20, where appreciable fungus infection is the limiting factor. While it is undoubtedly true that fungi will not grow in dry sugar, nevertheless the moisture required to stimulate their activity is remarkably slight in amount. The nature and extent of the fungus infection are indubitably predisposing factors in any sensible deterioration of sugar. In the light of these researches, then, it would be deemed advisable to revise our conception of the factor of safety rule in such fashion as to make adequate provision for fungus infection, which appears more destructive than any other single group of microorganisms in sugar yet studied. Experiments are now in progress to define more rigorously the limits of concentration for the activity of these fungi and the results will be available in the near future.

Before commencing the chemical analyses indicated in Tables XII-XIV a mycological examination was made by plating out a portion of the contents of each flask. Ten gm. portions were dissolved in 40 cc. of 17° Brix sugar solution, 1 cc. being plated on Kopeloff's agar, and incubated at 30°C. for 5 days. It will be seen from Table XV that all the inoculated flasks of the

TABLE XV.

MYCOLOGICAL EXAMINATION OF INOCULATED SUGARS.

PLANTATION GRANULATED.

After One Month Incubation.

FUNGUS	Dup.	Dup.	Average	Average Per Gm.	Average Per Gm. Original Inoculation
Check	2X	2X	2X	8X	0
Aspergillus flavus	118	136	127	638	1000
Blue Aspergillus	766	800	873	3915	10,000
Syncephalastrum	N	N	N	N (3X)	40
Aspergillus niger	1033	933	983	4916	8,000

X—Contaminating organism.
N—Covers plate.

Plantation granulated series indicated the presence of an abundant number of colonies of the fungus used. The average ranges from 638 to 4916 per gm. The check flasks show a negligible contamination. The original inoculation recorded in the last column represents the number of spores found by using the Zeiss blood-counting cell[23]. Obviously the direct microscopic count is always higher than the plate count. This has been clearly demonstrated in the work of Conn[8]. Therefore the apparent discrepancy between the original and final count is quite negligible. One outstanding fact, however, is that there is no indication of multiplication on any large scale. The same general results were obtained

TABLE XVI.

MYCOLOGICAL EXAMINATION OF INOCULATED SUGARS.

REFINED.

After One Month Incubation.

FUNGUS	Dup.	Dup.	Average	Average Per Gm.	Average Per Gm. Original Inoculation
Check	0	0	0	0	0
Aspergillus flavus	102	100	101	505	1000
Blue Aspergillus	800	966	883	4115	10,000
Syncephalastrum	N	N	N	N	40
Aspergillus niger	316	316	316	1580	8,000

X—Contaminating organism.
N—Covers plate.

with the Refined series as shown in Table XVI. The number of colonies found is in close agreement with the count made in the previous series, the only marked difference occurring with *Aspergillus niger*. Again there is no evidence of any considerable multiplication.

Table XVII.

MYCOLOGICAL EXAMINATION OF INOCULATED SUGARS.

CUBAN RAW.

After One Month Incubation.

FUNGUS	Dup.	Dup.	Average	Average Per Gm.	Average Per Gm. Original Inoculation
Check	2X	3X	3X	15X	0
Aspergillus flavus	11	9	10	52	13,300
Blue Aspergillus	0	0	0	0	64,000
Syncephalastrum	N	X	X	5X	8,000
Aspergillus niger	246	280	263	1315	38,400

X—Contaminating organisms.
N—Covers plate.

The results obtained in the Cuban raw series are incorporated in Table XVII, and in general the count is much lower than in the previous sets. The most striking feature is the great reduction in numbers as compared with the original inoculation. If anywhere, it is in this series that the greatest number of organisms would be expected, because of the higher moisture content. Especially baffling is the fact that no colonies of *Blue Aspergillus* were to be found and the sugar in the flasks containing this fungus showed the greatest deterioration. Possibly some error in the plating was responsible, but, unfortunately, the flasks were exposed to contaminating influences before that operation could be repeated. As before, the direct microscopic count is significantly larger than the final plate count.

A microscopical examination was made of the contents of each flask, taking several 1 gm. portions. The authors were assisted in this by Dr. Charles Thom's visit to this laboratory. He not only made numerous microscopical examinations of inoculated and uninoculated samples of sugar, but was generous enough to go over all cultures of fungi mentioned in Table III, in addition to spending some time on the factory problems at hand. The presence of mycelia was established (by staining with Conn's[8] Rose Bengal) in several instances where deterioration had occurred (see Plates I and II). This is indicated by the plus and minus signs in the third column of Tables XII-XIV. Thus, in Table XII, deterioration occurred in flasks 7, 8

and 9 where mycelia were present, etc. In no case was the presence of mycelia unaccompanied by evidence of deterioration. However, in other instances where deterioration was noted no mycelia could be detected, and only spores had been present. Such is the case in Table XIV, flasks 7, 8 and 9, where the greatest amount of deterioration in any one series is recorded.

This phenomenon is worthy of further study (which is at present being carried forward*), for upon its interpretation depends much that is of economic significance. Since it is possible for the spores of fungi to secrete enough invertase to cause the deterioration of sugar without the development of mycelia, then sugar which has been properly dried and considered "safe" by virtue of its moisture ratio, would in reality be likely to undergo deterioration, depending upon the nature and extent of the infection. The fact that fungus spores alone may cause deterioration points indubitably, from a new angle, to the necessity for cleanliness in the sugar mill. It leads to an investigation now in progress of sterilizing sugar in the centrifugals or reducing the infection by adequate protection at this point. From the broader viewpoint, it is of prime importance to industrial mycology to have determined to what extent the spores of fungi are capable of secreting enzymes without active growth, and it is hoped that the experiments now being conducted in this laboratory will throw some light on this question. For the present it may serve to interpret some interesting phenomena. For example, in a recent conversation Dr. C. A. Browne has suggested that it appeared to be a plausible explanation of the fact which he has often noted, that some sugars which start to deteriorate at a high moisture ratio, continue to do so in spite of the lowering of this ratio below the critical point of safety. Similarly, Dr. C. L. Alsberg regarded such an interpretation as bearing on the yield of penicillic acid under anærobic conditions by an ærobic fungus, *Penicillium puberulum*[1]. Instances could be multiplied indicating the application of this phenomenon to other scientific and industrial fields. The bearing which it might have upon the factor of safety rule is of more immediate concern. It has been

* While awaiting publication, we have succeeded in establishing experimentally that the spores of *Aspergillus niger* contain invertase, by inoculating 10 and 20 per cent sugar solutions with spores alone and obtaining a 5 per cent reduction in polarization with 10 cc. of heated spore suspension, and 10 per cent reduction with double this number of spores (i. e., 20 cc.) in 24 hours. There was a proportional increase in reducing sugars.

previously mentioned in connection with the earlier inoculation experiments that the moisture ratio for inhibiting fungus activity was found to be considerably lower than for other microorganisms. No mycelia were found in the sugars analyzed in Tables VII-IX; therefore, it is reasonable to suppose that the spores were responsible for whatever slight deterioration was observed. It becomes more imperative than ever, in the light of these data, to consider a revision of the factor of safety rule from the biological standpoint of fungus infection.

PART III.

FUNGI IN THE SUGAR FACTORY.

Having isolated the fungi which occur in marketable cane sugars and determined their power to cause deterioration by inversion, it was of practical significance to trace the fate of the fungi and bacteria through the process of sugar manufacture. With this in view, a daily bacteriological and mycological examination of each stage in the process was carried on throughout the past grinding season. In brief, the local process consists of crushing the harvested cane to extract the raw juice. This is treated with sulfur dioxide fumes and later limed back to slight acidity and brought to a boil. The clear juice resulting from this clarification is, after settling, run into a storage tank. The solid impurities are put through a filterpress—the filtered juice joining the clarified juice in the storage tank. The juice is evaporated to syrup in the "effects" by heating at about 120-185°F., under a vacuum of 10-26 inches of mercury. The syrup is taken to the "vacuum pan," where the sugar is grained at 130-165°F., under a vacuum of 22-25 inches of mercury. The massecuite, as usual, is run into the centrifugal and washed. Further methods of refinement, other than the use of vegetable carbon experimentally, were not employed in our sugar mill.

The samples were collected in sterilized, cotton-plugged, 300 cc. Erlenmeyer flasks and the effort was made to complete the plating within 1 hour of their arrival to the laboratory, the samples being kept in the refrigerator until used. A sterilized 18° Brix sugar solution was used as dilution water throughout. One cc. of each of the following dilutions was plated in duplicate on Kopeloff's agar (Czapek was used for comparison in the last runs): No dilution, Dilution A, 20 cc. in 180 cc. of dilution water constituting 1-10 dilution; Dilution B, 10 cc. of A in 90 cc. constituting 1-100 dilution; Dilution C, 10 cc. of B in 90 cc. constituting 1-1000. Sterilized 10 cc. and 1 cc. pipettes were used in the usual manner.

In Tables XVIII-XXIII are presented the averages of the duplicate counts for each dilution, each table representing what is known as a "run," and includes 5 "clarifiers." Obviously, the clarifiers lose their identity in the vacuum pan and only one

set of figures is obtainable for the succeeding stages in each run. In order not to further complicate the data presented, the average of Dilutions A, B and C is represented by a single column "Dil." It must be stated that the agreement between these dilutions was not all that could be desired, but it was natural to expect wide discrepancies where such a concentrated material was under observation. The data as presented give an adequate cross-section of what may be found in a small Louisiana mill. No attempt was made to classify the bacteria found, but they may be said to approximate qualitatively and quantitatively the species described by Owen[29]. (In fact, the heading "Bacteria" includes all microorganisms other than fungi.)

Table XVIII.

MICROORGANISMS IN THE FACTORY.

Run 1.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
2	No. Dil.....	NS NAf	7S	0	22BA	0	38BA 60C1 5II 3Ci	NC1 12S	NC1 BA Af	160C1 50BA 3II	10BA	0	14C1 7BA	17C1
	Dil.....	1000S 2300 Af 50T	10S 40Af	0	30BA	0	280BA 115C1 400Ci	400BA 310C1		200C1 50BA 30P				
	Bact.....	*1000	*70	5	*28	*10	2200	340	?	400	500	175	33	10
3	No. Dil.....	8BA 7T 3III	0	0	0	BA Ci	BA							
	Dil.....	4300T 1300BA 1000II	0	0	0	10 BA	0							
	Bact.....	*400	*30	65	N	N	19							

4	No. Dil.....	S,T	0	0	S	0	0								
	Dil.....	55S 250BA	0	0	0	0									
	Bact.....	*800	1200	0	N	N	225								
5	No. Dil.....	29BA 9S	S	0	3S	2S									
	Dil.....	2500BA 10S	0	0	0	0									
	Bact.....	*400	190	220	350	380									
Av. No. Dil.....		9BA 27S 2T	2S	0	5BA,S	0									
Av. Dil.....		269S 1087BA 1087T 250 II 575Af	3S 10Af	0	7BA	2BA									
Av. Bact.....		*650	*25	73	*14	5190	814								

Table XIX.

MICROORGANISMS IN THE FACTORY.

Run 2.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
1	No. Dil.	0	0		0		0	0	31C1 2BA S	230C1 BA S	3BA 7C1	0	BA	C1 BA S Af. Ci.
	Dil.	0	0		0		0	0	100C1 10BA 10S	0	80C1 10BA	0		
	Bact.	*800	*7		225		200	180	85	800	110	2500	4	14
2	No. Dil.	0	0		0		0							
	Dil.	0	0		0		0							
	Bact.	*1500	?		40									
3	No. Dil.	0	S		0									
	Dil.	25S												
	Bact.	*270	?		*12									
4	No. Dil.	0	0											
	Dil.	0	0											
	Bact.	*1000	3300		770									
5	No. Dil.	0	2S											
	Dil.	105S	425BA											
	Bact.	N	N											
Av. No. Dil.		0	S	0										
Av. Dil.		26S	85BA											
Av. Bact.		*792	5150											

TABLE XX.

MICROORGANISMS IN THE FACTORY.

Run 3.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR					
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed	Molasses	Wash Water	Cent. Air Above	Cent. Air Below
1	No. Dil.....	0	0	0	0	0	Or. 2C1	S 4BA 5C1	S 37C1	S 2BA 12C1	15C1 20 2BA	Or. 0	5C1	S 10C1
	Dil.....	0	0	0	0	0	0	40BA 30C1	10S 70C1 30BA	10S 60C1	110C1 30BA	0	0	0
	Bact.....	N	N	30	19	270	80	275	65	120	270	1600	14	180
2	No. Dil.....	0	BA	0										
	Dil.....	0	15BA	0										
	Bact.....	*1000	2250	220										
3	No. Dil.....	25BA 22Ci	2BA	0	Or.	C1 P	S BA							
	Dil.....	850BA	0	0	0	0	10BA 10S							
	Bact.....	*1000	5000	N	290	10	100							

Table XX—Continued.

MICROORGANISMS IN THE FACTORY.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
4	No. Dil.....	18BA S 3Ci	0	S Af.	S 3P	0								
	Dil.....	1200BA 10S	0		0	0	0							
	Bact.....	*220	430		90	130	5000							
5	No. Dil.....	8BA	4S	10S	0	BA	BA C1							
	Dil.....	3700BA 4000T	10S	0	0	10BA	0							
	Bact.....	*425	4000	*10	5500	60	130							
Av. No. Dil.....		10BA 5Ci	S BA	2S	0	P	C1 BA							
Av. Dil.....		1150BA 2S 800T	2S 4BA	0	0	2BA	2BA 2S							
Av. Bact.....		*661	3143	3417	1475	118	1327							

Table XXI.

MICROORGANISMS IN THE FACTORY.

Run 4.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
1	No. Dil.....	2S 11BA	5S	0	S	S	0	S	4An. NS	4An. 3S	0	0	3Af.	4C1 Or. Syn.
	Dil.....	100S 100BA *3Af.	10BA 20S	0	10S	0	0	10S	35S	10An. 20BA 10S	0	0	0	0
	Bact.....	*400	8500	80	400	550	180	1000	460	310	1730	750	2	8
2	No. Dil.....	NAf.	S	0	0	0	0							—
	Dil.....	310Af.	0	0	0	0	0							—
	Bact.....	*1000	8000	50	20	55	55							—
3	No. Dil.....	8S NAf. 6BA	0	0	0	0	0							
	Dil.....	*3Af. 500S	0	0	0	0	0							
	Bact.....	*800	0	5	*10	1120	60							

Table XXI—Continued.

MICROORGANISMS IN THE FACTORY.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
4	No. Dil.....	NAf. 4S	0	0	0	0	0							
	Dil.....	2500Af. 20S 100Ci	0	0	0	0	0							
	Bact.....	*750	1200	35	*7	12	100							
5	No. Dil.....	NAf. 5S	0	0	0	5Pd.	0							
	Dil.....	*2Af. 1100S *2Or.	0	0	0	0	0							
	Bact.....	*1000	*4	40	68	0	6							
Av. No. Dil.....		4S 3BA NAf.	2S	0	S	Pd.	0							
Av. Dil.....		344S 25BA 2200Af. 25Ci 400Or.	4S 2BA	0	2S	0	0							
Av. Bact.....		*790	4300	42	3500	346	80							

TABLE XXII.

MICROORGANISMS IN THE FACTORY.

Run 5.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
1	No. Dil.....	6Af. 2BA 5Pd. S					0	0	7S	2S	S	0	An. P Ci	Af. 2Ci 1S 3An. 2P 20C1
	Dil.....	1500Af. 70S 120BA 100An.					0	0	0	0	0	0	0	0
	Bact.....	*123					430	2200	1400	1450	*9	200	3	7
2	No. Dil.....	8S 15BA NAf.	0		0	0								
	Dil.....	1000S 200BA *8Af.	0		0	0								
	Bact.....	*300	1300		0	0	0							
3	No. Dil.....	NAf. An. BA S, T					0	0						
	Dil.....	150S 2200Af. 800BA 200Ar.					80							
	Bact.....	*1000												

Table XXII—Continued.

MICROORGANISMS IN THE FACTORY.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
4	No. Dil.....	0	0		0		0							
	Dil.....	0	0		0		0							
	Bact.....	*1000	1900		700		160							
5	No. Dil.....	5S NAf.	9S		0		0							
	Dil.....	2000S 2000Af.	60Af. 10S		0		0							
	Bact.....	*1500	5000		1500		600							
Av. No. Dil.....		NAf. 4BA 3S	3S		0		0							
Av. Dil.....		2640Af 645S 229BA 25An. 50Ar.	20Af. 3S		0		0							
Av. Bact.....		*800	2730		733		254							

Table XXIII.

MICROORGANISMS IN THE FACTORY.

Run 6.

Clarifier	FUNGI	JUICES					Syrup	⅔ SYRUP AND ⅓ MOLASSES				Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled		Masse cuite	SUGAR Raw	SUGAR Washed	SUGAR Molasses		Above	Below
1	No. Dil.....	NAf. 20S	0	0	0	0	0	S	BA	3An. 8Cl BA	P S			
	Dil.....	*5Af. *2BA	0	0	0	0	0							
	Bact.....	*600	2100	10	190		120	600	73	30	175			
2	No. Dil.....	NAf. 2P 10Ar.	2S	0	0		2An.							
	Dil.....	2500Af. 500S 1100 III	10S				10An.							
	Bact.....	*600	4500	200	900		1250							
3	No. Dil.....	NAf. NS												
	Dil.....	*2Af. *3S												
	Bact.....	*800	*10	6	35		130							

Table XXIII—Continued.

MICROORGANISMS IN THE FACTORY.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
4	No. Dil.....	NAf. NS	NS	0	0		0							
	Dil.....	*3Af. *8S	0	0	0		0							
	Bact.....	*1000	*7	5	620		750							
Av. No. Dil.....		NAf. NS 2Ar.	S	0	0		0							
Av. Dil.....		3100Af. 500BA 220,III	2S				2An.							
Av. Bact.....		*750	5900	55	438		562							

KEY: *—Thousands omitted.
N—Too numerous to count.
Af—Aspergillus flavus.
An—Aspergillus niger.
Ar—Aspergillus repens.
BA—Blue Aspergillus.
Cl—Cladosporium.
Ci—Citromyces.
Or—Sterile Orange Fungus.
P—Penicillium.
Pd—Penicillium divarticatum.
S—Syncephalastrum.
T—Trichoderma.
I, II, III—Unknown Sterile I, II, III.

In discussing the results presented in Tables XVIII-XXIII, it will be seen that Run 1 had perhaps the greatest amount of fungus contamination. This would be expected because of the fact that the mill had not been in operation for about one year prior to this time. The *Blue Aspergillus* appears with greatest frequency, both qualitatively and quantitatively, in this run and maintains the same tendency throughout the remainder of the season. This is especially significant since it links up with the chain of evidence already gathered which indicates that this fungus is not only found more frequently than any other in sugars of various types but has, moreover, a greater deteriorative power. While the fungus flora of each factory depends necessarily upon a variety of factors, nevertheless we are convinced that even though the *Blue Aspergillus* may not be the predominant organism in every factory, it is present universally. For example, we have found it in two of the largest and cleanest factories in Louisiana. Waksman[44] corroborates the work of Werkenthin[46], which indicates that the *Aspergilli* are predominant in southern soils. Noel Deerr in a recent correspondence suggests that it is reasonable to suppose that fungus infection from the soil may accompany the cane to the mill, and that such infection may be further disseminated by the boots of field workers, etc. This is in accordance with our observations, and represents a factor worthy of serious consideration. It may be added in this connection that Dr. Thom has found an *Aspergillus* to be the main agent in the destruction of certain highly concentrated foodstuffs. *Cladosporium*, likewise, is prevalent under the local conditions, and it is to be found not only through the data in the tables under consideration, but also in abundance on the woodwork, walls, floors, etc., of the mill. We have found the drip-board of the filterpress literally covered with this organism and the *Sterile Orange* fungus. *Syncephalastrum* has appeared somewhat more frequently than was to be expected.

Run 2, shown in Table XIX, had 1 per cent of rice hull carbon added (based on the weight of the juice). It is difficult to account for the absence of fungi in the raw juice of this run, as well as the first 2 clarifiers of Run 3, as shown in Table XX, and no explanation has suggested itself beyond the possibility of some unconscious error in technique.

In Run 5, which received 0.7 per cent rice hull carbon, it will be observed that no fungi appeared in the filtered and settled juices. It is altogether reasonable to suppose that the finely divided carbon mechanically removed some of the microorganisms. Beginning with clarifier 3 of this series, Czapek's agar was used to the end of the season, the results comparing favorably with those previously obtained on Kopeloff's modification, which required a shorter incubation period.

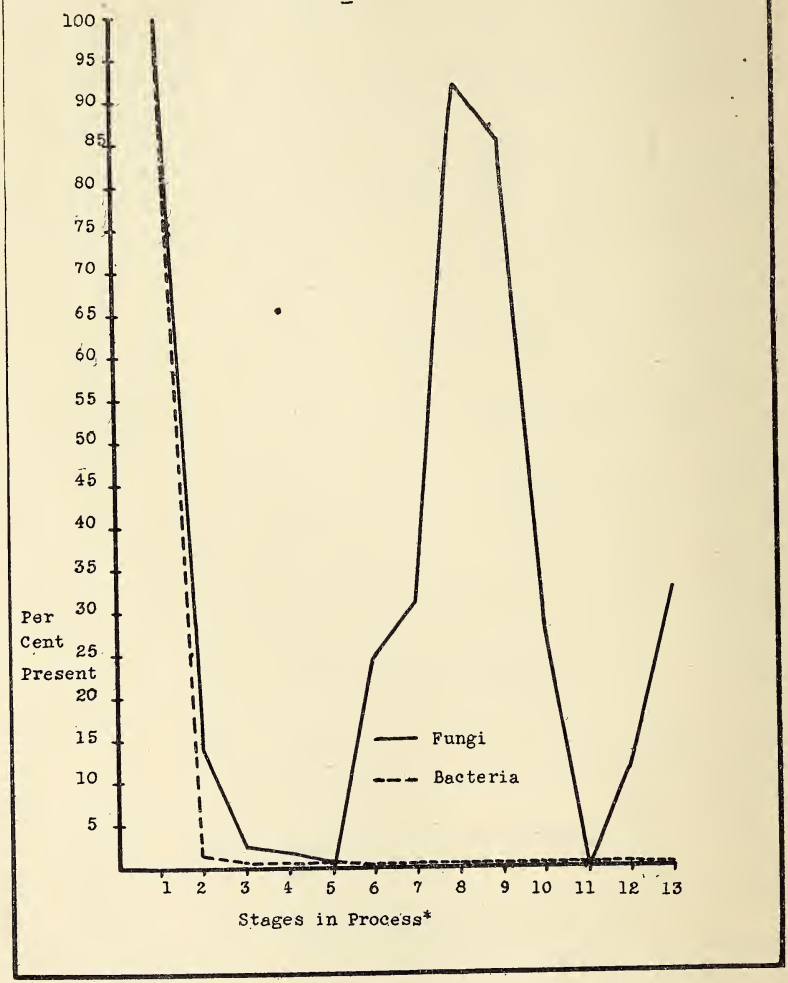
In Run 6, shown in Table XXIII, the syrup of clarifiers 1-5 was augmented in the vacuum pan by the addition of molasses (⅓) taken from previous runs. The results are similar to those recorded in other runs.

Table XXIV.

SUMMARY OF MICROORGANISMS IN THE FACTORY.

RUN		JUICE					Syrup	Masse-cuite	Raw Sugar	Washed Sugar	Molasses	Wash Water	CENT. AIR	
		Raw	Sulph.	Limed	Filt.	Settled							Above	Below
1	Fungi	15	1	0	5	0	53	61	51	100	5	0	11	9
	Bact.	100	3.9	.01	2.2	0.8	0.4	.05	?	.06	.07	.003	.0005	.0001
2	Fungi	0	0		0		0	0	5	100	5	0	.001	.02
	Bact.	100	.71		.034		.028	.026	.011	.112	.013	.24	.0005	.0015
3	Fungi	50	40	5	0		35	32	100	40	50	0	15	15
	Bact.	100	.45	.50	.18	.20	.001	.042	.01	.02	.042	.26	.002	.027
4	Fungi	100	4	2	0	0	0	2	100	15	0	0	6	16
	Bact.	100	.61	.006	.47	.05	.012	.13	.062	.043	.23	.10	.002	.001
5	Fungi	100	4		0		0	0	14	4	2	0	6	58
	Bact.	100	.35		.09	0.3	.05	.29	.17	.17	1.1	.02	.000	.008
6	Fungi	100	1	0	0	0	1							
	Bact.	100	0.8	.007	.08	.07								
Ave.	Fungi	100	13.6	3.3	1.6	0	24.6	31.1	91.8	85.2	28.0	0	11.5	32.8
	Bact.	100	1.14	0.17	0.51	0.28	0.10	0.11	.06	.08	.27	.13	.001	.006

FIG. I



*Stages in Process.

1. Raw Juice.
2. Sulfured Juice.
3. Limed Juice.
4. Filtered Juice.
5. Settled Juice.
6. Syrup.
7. Massecuite.
8. Raw Sugar.
9. Washed Sugar.
10. Molasses.
11. Wash Water.
12. Air above Centrifugal.
13. Air below Centrifugal.

The data in Tables XVIII-XXIII are summarized in Table XXIV, where relative percentages are recorded. Thus the maximum number of organisms appearing in any stage is taken as 100, and the number appearing in the other stages calculated on this basis. A graph of these results is shown in Fig. 1. It is at once evident that the greatest number of microorganisms occurs in the raw or normal juice. The sulfitation causes a decrease of 86 per cent of fungi, while the completion of the clarification process renders the juice practically sterile. However, when the syrup and massecuite are exposed to the air, and especially when the latter enters the centrifugal, reinfection takes place. The fungus content of the syrup appears to be higher than might be expected, but it will be noted that the infection from Run 1, already discussed, served to unduly weight the average. The rapidly whirling centrifugal sucks air from below at great speed and any microorganisms on the floor or in the mill at large have an opportunity of gaining an entrance. This is corroborated by the fact that the air below the centrifugal has approximately 3 times the number of fungi to be found in the air above the centrifugal. The bacterial counts (including microorganisms other than fungi) follow the same general trend as the fungi. There is a greater reduction in numbers, through sulfitation, there being a reduction of 99 per cent, while the clarification process renders the juice practically sterile. The subsequent reinfection does not seem to be as serious as in the case of the fungi. While these results confirm similar ones reported by Owen[29], nevertheless the fact that we were not employing a strictly bacterial medium may account for slight differences.

It is to be regretted that it was impossible at this time to conduct similar exhaustive surveys of the fungus and bacterial flora in each stage of the manufacturing process in other mills, for the limitations attending the observations under the local conditions are clearly recognized.

PART IV.

PRACTICAL DEDUCTIONS.

At the outset of this bulletin the economic significance of the problem at hand was indicated. The fact that the deterioration of sugar in transportation, handling and storage is a result of biological agencies has never been questioned. Therefore, the paucity of extensive research made it advisable to undertake a study of the presence and activity of the fungi, a group of microorganisms which appeared likely to prove of considerable importance. A survey of a large variety of sugars indicated the presence of fungi in significant numbers. The Standard granulated sugars were comparatively free from fungi and infection seemed to be correlated with an increasing moisture content, the lower grades of sugar carrying the largest number and variety of fungi. As has been pointed out by other investigators, the keeping quality of a sugar depends upon moisture as one of its controlling factors. It is of prime importance, then, to reduce the moisture content as much as possible and avoid the subsequent access of moisture.

Since it has been shown that reinfection takes place on a large scale after the massecuite leaves the vacuum pan, it would be advisable to cover the mixer which conveys the massecuite to the centrifugals. This might very easily be accomplished by means of an adjustable and removable canvas or tarpaulin. In this way all dirt, dust and foreign matter, as well as microorganisms, could be excluded, and one of the sources of infection removed. Since the principal infection occurs at the centrifugal and before the sugar is bagged, it is of practical importance to take special precautions at this point. Cleanliness in the factory has been shown to be not only desirable but profitable. The returns are not questionable, but may be measured in dollars and cents. So long as the deterioration of sugar was ascribed to the activity of bacteria alone it was difficult to explain the beneficial effects of cleanliness in the factory, owing to the extreme resistance to heat, etc., of the spores. Owen[32] raises the question: "How, then, could cleanliness eliminate the causative agencies in sugar deterioration?" This query was answered by the discovery of the presence of fungi in sugar which have greater de-

teriorative powers than bacteria, but which have a low thermal death point and powers of resistance[43], and consequently can be successfully eliminated. And it is in and about the centrifugals that cleanliness is of paramount importance. If possible, the centrifugals should be in a room instead of in the open factory. Whether this is done or not it is essential that there be a concrete floor below the centrifugals. This should be kept clean throughout the day and a swabbing with a hot 0.5 per cent solution of formaldehyde would do much towards destroying contaminating influences. In the off season it would be decidedly advisable to sterilize the mill thoroughly. Where water is used in washing sugar, strict regard must be paid to its purity, for, as is often the case, where the water is contaminated with microorganisms, such a practice is equivalent to an inoculation of sugar with deteriorative organisms. There is no question but that closed centrifugals would be advantageous.

The possibility of actually sterilizing the sugar and washing by means of superheated steam, etc., in the centrifugals is now being studied. That the handling of sugar after it leaves the centrifugal requires care is self-evident, and the condition of the bags must not be disregarded in any consideration of infection. The proper storage of sugar requires a cool, dry, well-ventilated warehouse, preferably with a concrete floor, the sugar being piled on a false floor of boards to permit a thorough circulation of air. It is necessary to guard against all sources of contamination, thereby reducing the infection, which means the elimination of one of the principal factors in deterioration. With the increased interest in the results of scientific experimentation manifested by practical men, it is hoped that some progress will be made towards improving the keeping quality of sugar by insisting upon cleanliness in the factory and maintaining suitable conditions for storage. While the data presented in this bulletin are somewhat technical in character, nevertheless the results have been interpreted as above, from the practical standpoint, and the primary interest has always been in the application of these biological principles to the sugar industry.

In conclusion, the authors are appreciative of the assistance they have so generously received from: Dr. C. A. Browne, whose suggestions and advice have been invaluable in the inter-

pretation of much of the data; Mr. W. L. Owen, with whom frequent consultations brought material aid; Dr. Charles Thom, whose visit cleared up many difficulties; Assistant Director W. G. Taggart and Dr. F. W. Zerban, for their constant help; Mr. E. C. Freeland, for assistance in chemical analyses; Mr. J. McFetridge, of the American Sugar Refinery; Mr. J. C. Murphy and other members of the New Orleans Sugar Exchange; the Angola Plantation and other managements have very kindly supplied us with sugar for experimental purposes. The authors are grateful for the kindness of Dr. Foster M. Johns, of the Tulane University Medical School, in preparing the accompanying photographs, which required considerable time and painstaking effort.

SUMMARY.

1. The fungi isolated on a variety of media from a wide range of cane sugars belonged chiefly to the *Aspergilli* and *Penicillia*. *Aspergillus niger* and a *Blue Aspergillus* occurred in practically all samples.
2. A microscopic examination revealed fungus mycelium in some sugars.
3. Czapek's agar had the greatest relative efficiency of all the media studied for isolating a variety of fungi. The authors' modification showed to good advantage and was responsible for a more rapid colony development.
4. Sterilized sugars inoculated with pure cultures of fungi deteriorated rapidly where the moisture content was appreciable. Little, if any, deterioration occurred when the moisture content was reduced to a minimum.
5. The factor of safety for sugars well infected with fungi would appear to be lower than is generally supposed. Slight evidences of deterioration occurred in Plantation granulated sugar with a factor of 0.1 and in Cuban raw sugar with a factor of 0.20.
6. Fungi spores, as such, contain invertase. These same fungi were responsible for the inversion of sucrose where only spores were present, as well as where mycelia were developd.
7. The fungus which appeared with greatest frequency in all sugars, the *Blue Aspergillus,* also had the greatest deteriorative power.

8. Fungi and bacteria were traced through each stage of the manufacturing process and found in greatest numbers in the raw juice. The clarification process was in effect a sterilization. Reinfection took place in the massecuite and in and about the centrifugals.
9. The practical deductions involve cleanliness throughout the factory, especially in the vicinity of the centrifugals, and adequate storage facilities.

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